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# THE EFFECTS OF LIGHT, TEMPERATURE AND EXOGENOUS THYROXINE ON VISUAL PIGMENT COMPOSITION OF JUVENILE RAINBOW TROUT, *SALMO GAIRDNERI*

by

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# ABSTRACT

Rainbow trout (*Salmo gairdneri*) have a pair of visual pigments, a rhodopsin based on retinaldehyde (the aldehyde form of retinol, vitamin $A_1$) and a porphyropsin based on 3-dehydroretinaldehyde (the aldehyde form of 3-dehydroretinol, vitamin $A_2$). The proportion of these two visual pigments (commonly presented as percent porphyropsin) are known to change seasonally and may change in response to specific environmental factors such as light and temperature. In addition, exogenous thyroxine will induce an increase in the percent porphyropsin in rainbow trout.

Results from this study showed that fish kept under the designed laboratory light condition at a photoperiod of 12L/12D for one month had significantly higher percent porphyropsin than those held in total darkness (24D). Fish kept in low temperature (6° C) for a month had also significantly higher percent porphyropsin than those held in higher temperature (16° C). The light and temperature regimes to which the fish were subjected, rather than the initial percent porphyropsin, seemed to determine the visual pigment composition at the end of the experiments. When the fish were put into different temperatures (5° C, 10° C, 15° C; at 24D), new equilibria of visual pigment composition seemed to occur within a month. The final percent porphyropsin indicated by these new equilibria seemed to be closely related to the temperatures to which the fish were subjected. However, putting fish into different photoperiods (24L, 12L/12D, 24D; at 16° C) resulted in a continuous change of percent porphyropsin during the 45 days of experiment. In addition, the final

percent porphyropsin estimated on the 30th or 45th day of experiment did not seem to be proportional to the photoperiods to which the fish were subjected. Black and transparent eye caps were fitted to the two eyes of the same fish to test the unilateral effect of light on visual pigment composition of trout. A group of eight fish showed a significant differential between two eyes after they were subjected to the experimental condition (24L, 8° C) for a period of ten days.

Introduction of L-thyroxine to tank water (at a concentration of 10 µg/100 ml) significantly elevated both blood levels of thyroxine and percent porphyropsin in the retina of trout. Light (12L/12D) in comparison to total darkness (24D) also favored significantly higher percent porphyropsin in the thyroxine-treated fish.

Fish held in light (12L/12D) and high temperature (16° C) in comparison to those held in total darkness (24D) and low temperature (6° C) seemed to have higher level of percent 3-dehydroretinol (amount of 3-dehydroretinol relative to the total amount of retinol and 3-dehydroretinol) in blood. Certain data suggested that higher level of percent 3-dehydroretinol in blood might be associated with higher percent porphyropsin in the retina. Introduction of thyroxine to tank water significantly elevated the percent 3-dehydroretinol in blood. However, interpretation of the latter was impaired by the large variance and the small sample size in each group tested.

Discussion on the implications of the results on the role of light, temperature, thyroxine and 3-dehydroretinol to the fish is presented.

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TABLE OF CONTENTS

Chapter Page

INTRODUCTION . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 1

MATERIALS AND METHODS . . . . . . . . . . . . . . . . . . . . . . . . 8

MATERIALS AND EXPERIMENTAL PROTOCOL . . . . . . . . . . . . . . . . 8

Background Information on Fish . . . . . . . . . . . . . . . . . 8

Light and Temperature Control . . . . . . . . . . . . . . . . . 9

Effect of Light and Temperature on Visual Pigment Composition and Thyroxine Concentration in Blood - Experiments 1 and 2 . . . . . . . . . . . . . . . . 10

Change of Visual Pigment Composition over a Period of 45 Days - Experiments 3 and 4 . . . . . . . . . . . . . 11

Interrelationship of Levels of Thyroxine, Percent 3-Dehydroretinol in Blood and Visual Pigment Composition - Experiment 5 . . . . . . . . . . . . . . . . 12

Introduction of Thyroxine to Tank Water - Experiment 6 . . . 13

Capped-Eye Experiment - Experiment 7 . . . . . . . . . . . . . 15

METHODS . . . . . . . . . . . . . . . . . . . . . . . . . . . . . 16

Estimation of Percent $VP_2$ in the Retina . . . . . . . . . . 16

Thyroxine Assay . . . . . . . . . . . . . . . . . . . . . . . . 18

Assay for Percent 3-Dehydroretinol in Blood . . . . . . . . . 18

A. Extraction of retinol and 3-dehydroretinol . . . . . 19

B. Separation of retinol from 3-dehydroretinol in silicic acid column . . . . . . . . . . . . . . . . . . 19

C. Spectrofluorometric assay for concentration of retinol and 3-dehydroretinol . . . . . . . . . . . . . 20

Chapter Page

RESULTS . . . . . . . . . . . . . . . . . . . . . . . . . . . . 22

I. EFFECT OF LIGHT AND TEMPERATURE ON VISUAL PIGMENT PIGMENT COMPOSITION - EXPERIMENTS 1, 2, 3, 4, and 7 . . . . 22

II. EFFECT OF EXOGENOUS THYROXINE AND THE INFLUENCE OF PHYSIOLOGICAL LEVEL OF THYROXINE ON VISUAL PIGMENT COMPOSITION . . . . . . . . . . . . . . . . . . . . 33

III. RELATIONSHIP AMONG CIRCULATORY LEVEL OF THYROXINE, PERCENT 3-DEHYDRORETINOL IN BLOOD AND VISUAL PIGMENT COMPOSITION IN RETINA . . . . . . . . . . . . . . . . . . . 39

IV. OTHER RELATIONSHIPS . . . . . . . . . . . . . . . . . . 45

A. Body Weight Versus Thyroxine Concentration in Blood . . 45

B. Change in Optical Density Unit in Relation to Visual Pigment Composition . . . . . . . . . . . . . . 45

C. Effect of Bleeding on Thyroxine Concentration in Blood and Visual Pigment Composition in Retina . . . . 51

DISCUSSION . . . . . . . . . . . . . . . . . . . . . . . . . . 52

ROLE OF LIGHT . . . . . . . . . . . . . . . . . . . . . . . . 52

ROLE OF TEMPERATURE . . . . . . . . . . . . . . . . . . . . . 53

ROLE OF THYROXINE . . . . . . . . . . . . . . . . . . . . . . 55

ROLE OF 3-DEHYDRORETINOL . . . . . . . . . . . . . . . . . . 56

BIBLIOGRAPHY . . . . . . . . . . . . . . . . . . . . . . . . . 59

APPENDIX 1 . . . . . . . . . . . . . . . . . . . . . . . . . . 64

APPENDIX 2 . . . . . . . . . . . . . . . . . . . . . . . . . . 67

APPENDIX 3 . . . . . . . . . . . . . . . . . . . . . . . . . . 73

APPENDIX 4 . . . . . . . . . . . . . . . . . . . . . . . . . . 76

APPENDIX 5 . . . . . . . . . . . . . . . . . . . . . . . . . . 79

APPENDIX 6 . . . . . . . . . . . . . . . . . . . . . . . . . . 82

APPENDIX 7 . . . . . . . . . . . . . . . . . . . . . . . . . . 91

# LIST OF TABLES

Table Page

1. Result of factorial analysis of variance on data from Experiments 1 through 6 . . . . . . . . . . . . . . . . . . . . 26

2. Difference of molar percent $VP_2$ between two eyes of fish fitted with black and transparent eye caps . . . . . . . . 32

3. Mean values of percent 3-dehydroretinol and thyroxine concentration in blood at the end of Experiment 6 . . . . . . . 46

LIST OF FIGURES

Figure Page

1. The special adaptor designed to fit the microcuvette to the sample cell holder of the spectrofluorometer, Turner Model 430 . . . . . . . . . . . . . . . . . . . . . . 21

2. Visual pigment composition in fish at the beginning and at the end of Experiments 1 and 2 . . . . . . . . . . . . . . 24

3. Effect of light on the visual pigment composition over a period of 45 days . . . . . . . . . . . . . . . . . . . . . 29

4. Effect of temperature on the visual pigment composition over a period of 45 days . . . . . . . . . . . . . . . . . . . 31

5. Visual pigment composition and thyroxine concentration in fish with thyroxine introduced into tank water at a concentration of 10 μg/100 ml . . . . . . . . . . . . . . . 35

6. Thyroxine concentration in blood samples from fish in Experiments 1 and 2 . . . . . . . . . . . . . . . . . . . . 38

7. Visual pigment composition, thyroxine concentration and percent 3-dehydroretinol in blood sampled from fish in Experiment 5 . . . . . . . . . . . . . . . . . . . . . . . . 41

8. The relation of visual pigment composition to the percent 3-dehydroretinol in blood . . . . . . . . . . . . . . . . . . 44

9. The relation of thyroxine concentration in blood to the mean body weight of fish . . . . . . . . . . . . . . . . . . . 48

10. The relation of visual pigment composition to the amount of extracted visual pigment in the retina . . . . . . . . . . 50

# INTRODUCTION

Out of the five hundred or more species of fish whose visual pigments have been characterized, more than one-fifth possess a paired-pigment system (Ali and Wagner, 1975). A paired-pigment system means that in the retina of a species, rhodopsin and porphyropsin exist either simultaneously or sequentially. Rhodopsin and porphyropsin arise through the respective conjugation of 11-cis retinal[1] and 11-cis 3-dehydroretinal to a single type of opsin. The amount of porphyropsin relative to the amount of rhodopsin in the retina of the fish is, therefore, based on the relative proportion of 11-cis 3-dehydroretinal and 11-cis retinal in combination with the opsin. Different species of fish exhibit characteristic spectrophotometric absorbance maxima ($\lambda$max) for porphyropsins and rhodopsins, presumably owing to the differences in the amino acid sequence of the species-specific opsin molecules (Dartnall, 1957; Munz and McFarland, 1965).

Rainbow trout (*Salmo gairdneri*) are euryhaline fish possessing a paired-pigment system (Munz and Beatty, 1965). The absorbance maxima for their rhodopsin and porphyropsin are 503 nm and 527 nm respectively as determined by partial bleaching experiments (Munz and Beatty, 1965).

In many species with paired-pigment systems, the visual pigment composition, commonly given as percent $VP_2$ (from now on, meaning amount of porphyropsin relative to the amount of rhodopsin plus porphyropsin in

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[1] In this presentation, the following terminology will be used: vitamin $A_1$ alcohol = retinol; vitamin $A_2$ alcohol = 3-dehydroretinol; vitamin $A_1$ aldehyde = retinal = retinaldehyde; vitamin $A_2$ aldehyde = 3-dehydrotetinal = 3-dehydroretinaldehyde; rhodopsin = $VP_1$; porphyropsin = $VP_2$.

in the retina), has been noted to change under different environmental and physiological conditions. For example, in the sea lamprey, *Petromyzon marinus*, the transitions of visual pigment composition to predominately porphyropsin and to pure rhodopsin are probably associated with the respective upstream and downstream migrations (Crescitelli, 1956; Wald, 1957). For other euryhaline fishes, Beatty (1966) has found that ocean-caught coho salmon (*Oncorhynchus kisutch*) have mainly rhodopsin while porphyropsin predominates in the eyes from fish caught at the fresh-water spawning site. In several non-migratory species, it has been found that there are seasonal variations in the visual pigment composition (for reviews, see Bridges, 1972; Beatty, 1975).

Changes in the visual pigment composition of fish were first produced experimentally in rudd, *Scardinius erythrophthalmus* (Dartnall *et al.*, 1961). These investigators induced change in the visual pigment composition of rudd by holding fish in outdoor and indoor darkroom aquaria. Light favored rhodopsin and darkness, porphyropsin. Further experiments have since been conducted on this and other species of fish (Bridges, 1965; Beatty, 1966; Jacquest, 1969; Bridges and Yoshikami, 1970c; Allen, 1971; Allen and McFarland, 1973; Allen *et al.*, 1973). It turns out that the so-called "rudd effect," as Dartnall (1962) termed it, is not found in all paired-pigment species tested. For example, within the cyprinid family, the rudd respond to light (in comparison to darkness) by a decrease of percent $VP_2$ whereas the red-side shiner, *Richardsonius balteatus*, shows an increased proportion of porphyropsin (Allen, 1971).

In the salmonids, Dartnall (1962) reported that rainbow trout and

the brown trout (*Salmo fario = trutta*) also exhibit the rudd effect. Jacquest (1969) suggested that rainbow trout might respond to increased light with a decrease of percent $VP_2$. These results on salmonid fishes, however, are contradicted by the recent finding of Allen *et al.* (1973). They reported that out of three species of salmonid fishes they examined (rainbow trout; brook char, *Salvelinus fontinalis*; and brown trout), light favored an increase of percent $VP_2$ in two (rainbow trout and brook char) and exerted no significant effect for the other (brown trout). Thus the first objective of this study was to resolve some of the apparent discrepancies by investigating the effect of light on the visual pigment composition of juvenile rainbow trout.

One important environmental factor which may influence fishes both seasonally and during the spawning migration is temperature fluctuation. In spite of the studies on the effect of light on the change of visual pigment composition, no work has been done on temperature effects until recently (Allen and McFarland, 1973; Allen *et al.*, 1973). Allen and McFarland (1973) showed that low temperature (6° C) favored increased porphyropsin in juvenile golden shiner, *Notemigonus crysoleucas*. However, the study was impaired by the mortality of two groups of fish at high temperature (21° C). Allen *et al.* (1973) commented on the possible temperature effect on visual pigment composition of rainbow trout but unfortunately did not publish any supporting experimental evidence. In view of this incomplete picture so far published for a temperature effect, my second objective was to study the influence of temperature, in addition to that of light, on the visual pigment system of juvenile rainbow trout.

The research on the influence of thyroxine on visual pigment composition in fish was initiated by the study on rainbow trout (Munz and Swanson, 1965). Thyroxine was found to induce an increase of porphyropsin in the fish (Munz and Swanson, 1965). Later investigations showed that thyroid hormones increased porphyropsin in juvenile and mature kokanee salmon, *Oncorhynchus nerka* (Beatty, 1969, 1972), the red-side shiner (Allen, 1971), juvenile coho salmon (Beatty, 1972), rainbow trout (Jacquest and Beatty, 1972) and Atlantic salmon parr and smolt, *Salmo salar* (Beatty, 1975). However, when thyroxine was administered to the rudd (Bridges and Yoshikami, 1970b) and the rainbow smelt, *Osmerus eperlanus* (Bridges and Delisle, 1974), no significant change of visual pigment composition was found. The action of thyroxine, as revealed by these studies, needs further clarification for the following reasons:

1) The means of administration of thyroid hormones varied with different investigators. Some preferred intraperitoneal injections (Beatty, 1969, 1972; Jacquest and Beatty, 1972; Bridges and Delisle, 1974); others introduced thyroxine directly into the tank water (Munz and Swanson, 1965; Allen, 1971; Cristy, 1974; Bridges and Delisle, 1974) and still others unilaterally injected thyroxine into the ocular tissue (Bridges and Yoshikami, 1970b). None of these researchers measured the extent to which the level of thyroid hormones were altered in the fish in relation to the visual pigment composition. This might cause erroneous conclusions that thyroid hormones were not effective in changing visual pigment of a certain species while, in fact, there was no change in the amount of circulatory thyroid hormones in the fish.

2) The degree to which environmental factors and/or stages of maturation of fish influence the level of thyroid hormones has not been

fully investigated. Could thyroid hormones be that part of the neuroendocrine system through which environmental factors and/or sexual maturation exert their influence on the change of visual pigment composition in fishes?

3) Although light appears to have various effects in different paired-pigment fishes (for review, see Bridges, 1972), thyroxine seems effective only in increasing percent $VP_2$ (for review, see Beatty, 1975). Moreover, Beatty (1969) showed that its action is more marked when the kokanee were held in light or a light-dark cycle than when the fish were held in constant darkness. Therefore the interaction of light and thyroid hormones on visual pigment composition deserves further attention.

Cristy (1974) has induced changes of visual pigment composition in rainbow trout by intraperitoneal injections of prolactin. This might have resulted from prolactin-induced thyroxine release, a phenomenon which has been observed throughout the vertebrates (Etkin and Gona, 1974).

In view of the above, my third objective was to study the effect of exogenous thyroxine (by introducing it to tank water) on visual pigment composition of the rainbow trout.

The aldehyde forms of vitamin $A_1$ and $A_2$, which are essential for the synthesis of visual pigments, originate from the dehydrogenation (or oxidation) of the alcohol forms of the vitamins (for review, see Bridges, 1972). This dehydrogenation is thought to occur within the ocular tissue (Dartnall, 1964; Bridges, 1965). Therefore, the supply of retinol and 3-dehydroretinol to the ocular tissue is important for the synthesis of the visual pigments.

Retinol and 3-dehydroretinol are stored in the body of rainbow trout mainly in the liver and the pyloric caeca (Braekkan *et al.*, 1969). From mammalian studies (mainly clinical studies on the laboratory rat), it was found that vitamin A is carried from the liver to the ocular tissue through the circulatory system mainly in the form of all-trans retinol (Olson, 1969). Arriving at the retina and the pigment epithelium of the eye, all-trans retinol is either oxidized to all-trans retinal, isomerized to 11-cis retinal and conjugated to opsin for formation of new visual pigments or stored in the form of an ester (mainly retinyl palmitate) (Olson, 1969). In fish, studies have shown that the percent 3-dehydroretinol (from now on, meaning the amount of 3-dehydroretinol relative to the total amount of retinol and 3-dehydroretinol) in the pigment epithelium correlates with visual pigment composition in the retina (Wald, 1939; Bridges and Yoshikami, 1970c). However, similar correlations were not found between liver and pigment epithelium in fish (Wald, 1939; Bridges and Yoshikami, 1970c). The major link between the liver and the pigment epithelium, namely the circulatory system, has so far not been investigated for percent 3-dehydroretinol.

In rainbow trout and kokanee, administration of 3-dehydroretinol through intraperitoneal injection or the diet has been shown to significantly increase the percent $VP_2$ in retina (Beatty, 1972; Jacquest and Beatty, 1972). This implies that an elevation of percent 3-dehydroretinol in blood might change the visual pigment composition, suggesting that the normal (or physiological) percent 3-dehydroretinol in the blood might reflect the visual pigment composition in the retina. To investigate this suggestion, visual pigment composition and percent

3-dehydroretinol in blood from a number of fish subjected to different temperature and light regimes were examined.

The studies involving the effect of thyroid hormone and percent 3-dehydroretinol in blood are based on the assumption that visual pigment composition may be determined by some central control mechanism. However, Bridges and Yoshikami (1970b, c) proposed that the control mechanism for visual pigment composition in the rudd was located in the pigment epithelium. The most persuasive evidence to support this "local control mechanism" hypothesis was the induction of a differential in the visual pigment composition in the two eyes of the same fish. In addition, they could not demonstrate any change of visual pigment composition in rudd treated with thyroxine (Bridges and Yoshikami, 1970b, c). They achieved the bilateral differential in visual pigment composition by the elimination of light to one of the two eyes, using an opaque "eye-cap." A similar capped-eye experiment was undertaken for rainbow trout, a species proven to respond to exogenous thyroid hormone (Munz and Swanson, 1965; Jacquest and Beatty, 1972; Cristy, 1974) and light (Allen *et al.*, 1973).

# MATERIALS AND METHODS

## MATERIALS AND EXPERIMENTAL PROTOCOL

### Background Information on Fish

Juvenile rainbow trout, *Salmo gairdneri*, were obtained from the provincial government rearing station in Crammond, Alberta (Raven Rearing Station) from December, 1974, to February, 1975. They were all yearlings with body weights ranging from 34 to 139 grams. At the rearing station, they were held in shallow outdoor ponds at temperatures ranging from 4° C in the winter to 15.5° C in the summer (Jacquest, 1969). Since the ponds were never frozen over, the fish were exposed to the natural light regime throughout the year.

Immediately upon arrival at the university, the fish were placed in 55-liter circular aquamarine fiberglass tanks at the temperature of the water at the rearing station. The water temperature was adjusted overnight to the appropriate acclimation temperature for individual experiments (see below). The circular tanks were covered with green corrugated sheets of fiberglass (Filon). Recording from the remote probe of a spectroradiometer (SP4660, ISCO Model SR) at the water surface, the total radiant energy from fluorescent light sources was 50 $\mu w/cm^2$ over the range of 750-380 nm [as measured by the method of Allen (1971)]. The photoperiod of the room light was set to correspond to the outdoor photoperiod. Fish were fed the same food (Silver cup, Murray Elevators, Utah) used in the rearing station. They were not fed during the experiment.

## Light and Temperature Control

All fish were subjected to controlled amounts of light and regulated temperatures during the experiments. In the experiments, the fish were held in 120-liter aquamarine fiberglass tanks with dechlorinated water flowing through at a rate of 100 to 200 liters per hour. A white wooden lid (73 x 56 x 40 cm) was placed on top of each tank, with two 20 W G.E. cool white fluorescent tubes (F20-T12-CW) located on the ceiling of the lid. The photoperiod inside the tank was controlled by a timer (TORK). For the continuously dark tanks, a single 7.5 W red bulb was fixed at a similar position, allowing illumination at brief intervals for the purpose of cleaning and removal of any dead fish. Strips of hard rubber were nailed onto the lower outside edges of all lids to prevent any room light from entering tanks. For the dark tanks, recordings taken at the water surface with the remote probe of the spectroradiometer indicated no detectable readings over the range of 380 nm to 750 nm. The total radiant energy for the illuminated tanks, however, varied (among different tanks) from 86.8 to 50.5 $\mu w/cm^2$ over the range of 380 nm to 750 nm. The total radiant energy from the small red bulb gave very low readings (0.9-0.6 $\mu w/cm^2$ from 380 nm to 750 nm). These measurements were taken at the beginning and the end of the experimental period.

Water temperature was controlled by a thermoregulator (T3345-C series, Magnetic, Canlab). The thermoregulator was connected to a relay (Sargent-Welch) which controlled a solenoid mounted onto the warm or ambient water inlet to the tank. Cold water (4-6° C) constantly flowed into the tank and the desired temperature was attained by the blending in

of warmer water via the controlled solenoid. Tank water temperature was constant to within 0.1° C. This was further confirmed every twenty-four hours during the experiment with a YSI telethermometer (Yellow Spring Instrument Co., Yellow Spring, Ohio).

### Effect of Light and Temperature on Visual Pigment Composition and Thyroxine Concentration in Blood--Experiments 1 and 2

Before the conditions for the subsequent experiments (Experiments 1-7) in this study were chosen, a number of pilot experiments were performed using different durations of experimental period, temperatures, and photoperiods. Owing to the small sample size and high mortality in each of these preliminary experiments, the details will not be presented here. Some of these results, however, are presented in Appendix 1.

Experiment 1 involved a total of 56 fish. They were preconditioned in two 120-liter aquamarine fiberglass tanks at 16° C in total darkness for approximately one month before being subjected to experimental conditions. At the beginning of the experiment fish were anaesthetized with tricaine methanesulfonate and the left eyes removed (see Appendix 1) under dim red light (Kodak safelight with Wratten Series 2 filter). In addition to enucleation, the operation included the recording of the wet weight of each fish, tagging (metal animal tag, number 1005 monel size 1, National Band and Tag Co., Kentucky; on the ventral portion of the caudal peduncle) and, except for five fish in one group (see below), withdrawal of approximately 0.3 ml of blood from the dorsal aorta. They were then divided into four groups and subjected to the following conditions for 28 days: 12L/12D at 16° C (group 1-A), 24D at 16° C (group 1-B), 12L/12D at 6° C (group 1-C) and 24D at 6° C (group 1-D). At the end of the

experiment, fish (except for those held in total darkness in the experiment) were dark-adapted for one (or one and a half) hour, anaesthetized and the right eyes removed under dim red light. Weight of fish was again determined, with blood samples withdrawn from the dorsal aorta of the fish. The sex of the fish was determined at autopsy by identification of the morphologically differentiated male and female gonads (Saunders and Manton, 1969). Blood and retinae of these fish were analysed for thyroxine concentration and visual pigment composition respectively (see later sections for methods). The numerical results from this experiment (and experiments hereon) are reported in Appendix 7.

Experiment 2 involved 61 fish. Within one to two days after arrival from the rearing station, these fish were dark-adapted and, except for five fish in one group which were not bled, were operated on in the same way as in Experiment 1. They were then divided into four groups: 24D at 6° C (group 2-W), 24D at 16° C (group 2-X), 12L/12D at 6° C (group 2-Y) and 12L/12D at 16° C (group 2-Z). At the end of 28 days, sampling was carried out as previously described.

Ten fish (five in group 2-Z and five in group 1-D) in the above two experiments were not bled at the beginning of the experiments so that the influence of bleeding on any change of visual pigment composition and blood thyroxine level could be estimated.

### Change of Visual Pigment Composition over a Period of 45 Days-- Experiments 3 and 4

Experiments 3 and 4, involving 171 fish, were designed to study individually the effect of light and temperature on changes of visual pigment composition over a period of 45 days. Fish were either operated

upon and then subjected to experimental conditions immediately upon arrival from the rearing station (88 fish, groups 3-L, 3-M and 3-N) or subjected to approximately one month of normal laboratory conditions at low temperature (4-5° C) before the experiment began (83 fish, groups 3-T, 3-U and 3-K). Fish were dark-adapted, anaesthetized, weighed, tagged and the left eyes removed at the beginning of the experiments. They were then subjected to one of the three experimental conditions for 45 days. Fish used in the experiment to test the effect of light (Experiment 3, fish not preconditioned) were divided into three groups of approximately 30 each. They were then held at 16° C in 12L/12D (group 3-L), 24L (group 3-M) and 24D (group 3-N). Fish used in the experiment to test the effect of temperature (Experiment 4, fish preconditioned at 4-5° C) were similarly divided into groups of approximately 30 each, held under constant darkness at 5° C (group 4-T), 10° C (group 4-U) and 15° C (group 4-K). Whenever possible, right eyes of two fish from each group were removed every three days. Wet weight and sex were determined after the fish were killed. Unfortunately, mortality was high for group 4-T (5° C, 24D) owing to failure of the water supply.

### Interrelationship of the Level of Thyroxine, Percent 3-Dehydroretinol in Blood and Visual Pigment Composition--Experiment 5

Although Experiments 1 and 2 would give information on how light and temperature may affect the thyroxine concentration in blood in relation to visual pigment composition, the amount of plasma obtained from those fish was insufficient to allow simultaneous determinations of thyroxine concentration and percent 3-dehydroretinol in blood from the same fish. Therefore a group of larger fish, with a mean body weight of

95.6 grams (n = 48, ranging from 77 g to 139 g), were selected (according to body weight) for this experiment. Upon arrival from the rearing station, they were dark-adapted, anaesthetized and the left eyes removed. Besides the routine weighing and tagging, 0.6 ml of blood was obtained from the dorsal aorta of each fish for the determination of percent 3-dehydroretinol in the blood. The fish were then divided into four groups of 12 each and subjected to one of the four different light and temperature conditions for 26 or 29 days:[1] 12L/12D, 16° C, 26 days (group 5-P); 12L/12D, 6° C, 29 days (group 5-Q); 24D, 16° C, 26 days (group 5-R) and 24D, 6° C, 29 days (group 5-S). Blood samples (0.8 to 1.2 ml from each fish) and right eyes were obtained at the end of the experiment. Both thyroxine concentration and percent 3-dehydroretinol were determined from the blood samples.

### Introduction of Thyroxine in Tank Water--Experiment 6

From the work of Eales (1974), it is assumed that a more stable and sustained increase of thyroxine level in blood can be induced by having thyroxine in the tank water. In this experiment, juvenile trout were subjected to 10 μg/100 ml (0.1 ppm) concentration of L-thyroxine (Nutritional Biochemical, Ohio) in static tank water. The general procedure followed that described by Eales (1974). L-thyroxine (T4) (0.12 g) was dissolved in 60 ml of 0.1 N NaOH solution and 5 ml of the thyroxine solution was introduced into the fish tank holding 100 liters of dechlorinated static water. Fresh stock thyroxine solution was prepared every five days. All groups (T4-treated and controls) of fish

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[1] This allowed the 3-dehydroretinol assay to be accomplished on all samples within 12 hours of withdrawal of blood from the fish.

were transferred every twenty-four hours to an identical tank (for T4 groups, containing freshly introduced T4). Temperature of these static tanks was held constant (at 8° C) by running cold water through a metal cooling coil immersed inside the tank. The cold water supply to the cooling coil was controlled by a solenoid connected to a relay and regulated by a thermoregulator as previously described.

Forty fish were preconditioned at 16° C in total darkness for one month. Due to mortality of unknown reason, only 33 fish survived the experiment. The operation at the beginning of the experiment was carried out in darkness under dim red light (Kodak safelight with Wratten series 2 filter). It included anaesthetizing the fish, removal of the left eyes, wet weighing and withdrawal of 0.5 ml of blood from the dorsal aorta of each fish. Fish were then divided into four groups and held at 8° C for 10 days under different experimental conditions: 24D, static tank water (group 6-E); 24D, static tank water with T4 introduced (group 6-F); 12L/12D, static tank water (group 6-G) and 12L/12D, static tank water with T4 introduced (group 6-H). Fungal disease was detected during the experiment, thus chloramphenicol (Chloromycetin® ; Parke, Davis and Co., Ltd., Ontario) was introduced (3 capsules per day per tank, 250 mg per capsule) to all tanks starting from the second day of the experiment. After dark acclimation, right eyes and blood samples were removed from the fish at the end of the experiment. In addition to thyroxine concentration, percent 3-dehydroretinol was also determined from the same blood samples.

## Capped-Eye Experiment--Experiment 7

A concave depression of approximately 5 mm deep was made onto an aluminum plate with a 25/64" (0.99 cm) drill. Eye caps were molded from a piece of transparent plastic (transparency normally used for overhead projectors, approximately 0.03 to 0.04 mm in thickness) by forcing the blunt end of a 10 x 75 mm pyrex test tube, preheated over a bunsen burner, into the concave depression of the aluminum plate, with the plastic lying in between the test tube and the aluminum plate. Transparent eye caps were simply made by cutting these concave depressions from the transparent plastic. Different sizes of caps can be manufactured simply by varying the size of the depression on the aluminum plate. Black caps were made by covering the transparent caps with a strip of black vinyl electrical tape (Scotch brand). In some instances, a drop of Krazy glue (alpha cyanoacrylate, Tel-Pro Products Ltd., Ontario) was applied to ensure binding of the black tape onto the transparent cap. The fish were anaesthetized with the eye caps fitted under room light. Several drops of Krazy glue were applied onto the junction where the tissue around the orbit met with the cap. To ensure the effectiveness of the glue, the area where the glue was to make contact had to be thoroughly dried. To hasten drying of the glue, a stream of air was applied to the surface of the applied Krazy glue for approximately 30 seconds. For fish fitted with two caps, the entire operation took two to three minutes.

Fish used in this experiment were preconditioned at 16° C in total darkness for approximately three weeks. At the beginning of the experiment, 17 fish were each fitted with one black and one transparent cap, three with two black caps and three with two transparent caps.

Fish were recapped if the caps dislodged from the eye within the first twelve hours. No more than six fish were placed in each of four tanks, with each individually marked by fin clips or metal tags in such a way that each could be identified at a distance. A black cross-mark was made on the transparent caps to facilitate the location of these caps if dislodged. A daily record was made on the condition of the caps on the eyes of each fish.

During this experiment which lasted for 10 days, the fish were maintained at 8° C and 24L. Inspection at the end of the experiment showed no cloudiness of the cornea of any capped eye. Mortality was high (30.4%) probably owing to the induced hypoxia during the capping.

## METHODS

### Estimation of Percent $VP_2$ in the Retina

Fish from most groups in the study were dark-adapted for one to one and a half hours before the eyes were removed from the orbit by a pair of forceps. This eye removal procedure, along with the dissection of retina and the extraction of visual pigments, was carried out under dim red light illumination (Kodak safelight with Wratten series 2 filter).

The eyes were placed in cold 4% alum solution (aluminum potassium sulfate) in light-tight canisters so that they could be transferred from one room to another. Dissection of the retina (for detailed procedures, see Jacquest, 1969) was done within twelve hours of eye removal. The retinae were then stored in darkness in 4% alum at -20° C for a period of up to 3 months before extraction. The visual pigments of each retina were extracted (for detailed procedures, see Munz and Beatty, 1965) with

0.5 ml of freshly prepared 2% digitonin (Nutritional Biochemical) solution. Saturated sodium borate solution (0.05 ml) was used to adjust the pH of the extract to about 8.5 before it was stored again at -20° C. A minimum of four days to a maximum of two weeks was allowed before the spectrophotometric analysis of extract was carried out.

For the detailed procedures and principles involving the bleaching experiment, one is referred to the published work by Dartnall (1957) and Munz and Beatty (1965). Neutralized solution (0.05 ml) of 0.02 M hydroxylamine ($NH_2OH$) was added to each extract before the initial and final absorbance spectrum of the extract was obtained from a Cary 14 recording spectrophotometer. The bleaching protocol consisted of a single ten-minute exposure of the extract to orange light (610 nm). Using a template curve prepared by Munz and Beatty (1965) from the pure difference spectra of $VP503_1$ (rhodopsin) and $VP527_2$ (porphyropsin) added in various proportions to represent mixtures, the percent $VP_2$ was determined from the wavelength at the 50% point of the total difference spectrum (for detailed procedures, see Appendix 2). Conversion of percent $VP_2$ (based on relative absorbance) to molar percent $VP_2$ (based on molecular concentration) was made using the values 40,600 and 30,000 as the molar extinction coefficients of rhodopsin and porphyropsin respectively[1] (see Dartnall, 1968). Statistical analyses were carried out on values of molar percent $VP_2$. Parametric tests were employed on the assumption that values of molar percent $VP_2$ of each group of fish are

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[1] From mathematical deduction, the following conversion formula was reached:

$$y = \frac{4.06x}{1.06x + 3},$$

normally distributed (Appendix 3). Significance is recognized at the 5% level in all tests.

## Thyroxine Assays

The determination of thyroxine concentration in blood samples followed the instructions supplied with the Tetralute $I^{125}$ Reagent Kit (Ames Company, Ontario) for the *in vitro* thyroxine assay. The method utilizes the principle of competitive protein binding (Murphy and Pattee, 1964) between thyroxine in the plasma sample and the added radioactive thyroxine with the thyroxine binding proteins in human plasma (Braverman *et al.*, 1971). This clinically oriented thyroxine kit has been proven to be useful in the study of circulatory thyroxine in the brook trout (*Salvelinus fontinalis*), rainbow trout and several other fresh-water species (Higgs and Eales, 1973). Rejuvenation of the sephadex columns used in the assay followed the method described by Higgs and Eales (1973). The blood samples, stored at -20° C, were analysed no later than 4 weeks after they were obtained from the fish.

## Assay for Percent 3-Dehydroretinol in Blood

The standard method for the determination of retinol and 3-dehydroretinol is known as Carr-Price test (Freed, 1966). It measures the concentration of the vitamins by a spectrophotometric method using antimony trichloride in chloroform to develop a blue colour (Freed, 1966). However, this test is insensitive for the amounts of the vitamins existing in small volumes of blood. Therefore, a spectro-

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where x = % porphyropsin expressed in the form of decimal figure, and
where y = molar % $VP_2$ expressed in the form of decimal figure.

fluorometric method had been adopted for the purpose of this study.

A. Extraction of retinol and 3-dehydroretinol.[1] The procedure for extracting the vitamins from blood was primarily based on the method by Kahan (1966) with modification from Thompson *et al.* (1971). The procedures employed in this study were as follows:

> 200 µl of plasma were placed in a 12 x 75 mm disposable culture tube and mixed with 300 µl of 99% ethanol by vortex mixing. 500 µl of petroleum ether (B.P. = 38 to 47°C) were then added and the mixture was thoroughly agitated (for 30 seconds by vortex mixer). Ten minutes were allowed for the vitamins to separate into the petroleum ether phase.

B. Separation of retinol from 3-dehydroretinol in silicic acid column. Separation of the vitamins was carried out in a silicic acid column (see Appendices 4 and 5). When the extracted vitamins (in petroleum ether) are added to a silicic acid column, retinol is retained by the column (Garry *et al.*, 1970). It can then be fully recovered by introducing an appropriate amount of isopropanol into the column (Garry *et al.*, 1970; see also Appendix 5). The 3-dehydroretinol, on the other hand, is fully recovered from the initial petroleum ether eluate (Appendix 5).

> The culture tube (containing plasma, alcohol and the extracted vitamins in petroleum ether) was centrifuged at 3000 XG for 5 minutes. 300 µl of the top petroleum ether layer were transferred onto the silicic acid microcolumn (for preparation, see Garry *et al.*, 1970). 600 µl of petroleum ether were then added to the column. The resulting eluate was then reduced to 400 µl (by evaporation under $N_2$) before assaying by fluorescence spectrophotometer. The column was then dried under $N_2$ and 800 µl of isopropanol were applied to the column. The isopropanol eluate was similarly adjusted to 400 µl before assaying.

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[1] All procedures from extraction to separation of the vitamins in silicic acid column were carried out under the illumination of a 40 or 60 W red bulb.

C. Spectrofluorometric assay for concentration of retinol and 3-dehydroretinol. The petroleum ether and isopropanol eluates were assayed for fluorescence at an emission wavelength of 490 nm and excitation wavelengths from 320 nm to 370 nm. A Turner model 430 spectrofluorometer (G. K. Turner Associates, California) equipped with an automatic wavelength drive was employed for this purpose. Owing to the minute volume of the eluates, they were assayed in a selected micro-cuvette instead of the normal borosilicate tubes (#1105-805, selected microcuvette, G. K. Turner Associates). A specially designed adapter (Fig. 1) was used to fit the microcuvette onto the sample tube holder in the sample compartment of the spectrofluorometer.

The fluorescence was recorded on a paper chart recorder (model MR, Sargent Welch) connected to the signal output of the spectrofluorometer. The fluorescence units were then converted to retinol and 3-dehydroretinol concentrations using a set of prepared standard curves (Appendix 6). The percent 3-dehydroretinol was then calculated from the concentrations of the two vitamins in the blood sample.

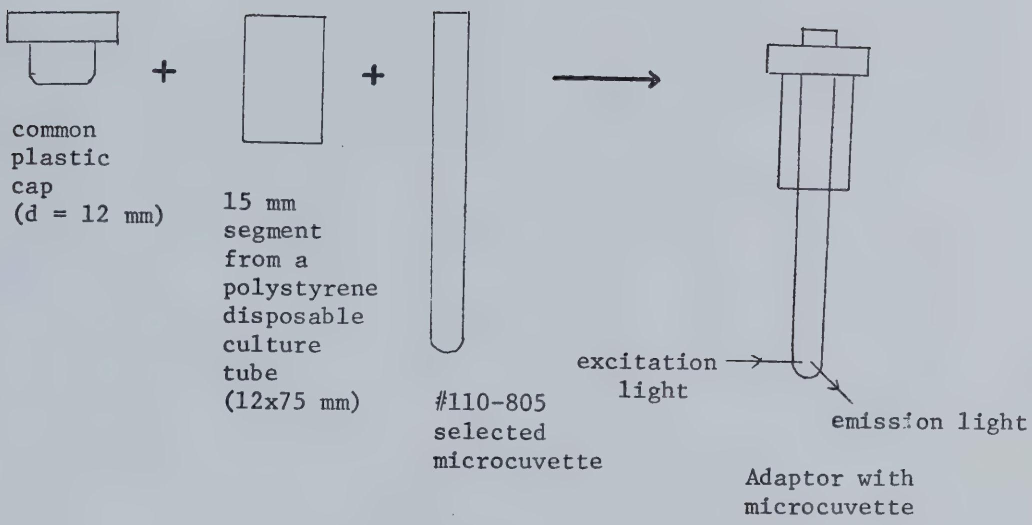

+
common plastic cap (d = 12 mm)
+
15 mm segment from a polystyrene disposable culture tube (12x75 mm)
#110-805 selected microcuvette
excitation light
emission light
Adaptor with microcuvette

Figure 1. The special adaptor designed to fit the microcuvette to the sample cell holder of the spectrofluorometer, Turner Model 430. (Figures are drawn to actual size.)

## RESULTS

### I. EFFECT OF LIGHT AND TEMPERATURE ON VISUAL PIGMENT COMPOSITION--EXPERIMENTS 1, 2, 3, 4 AND 7

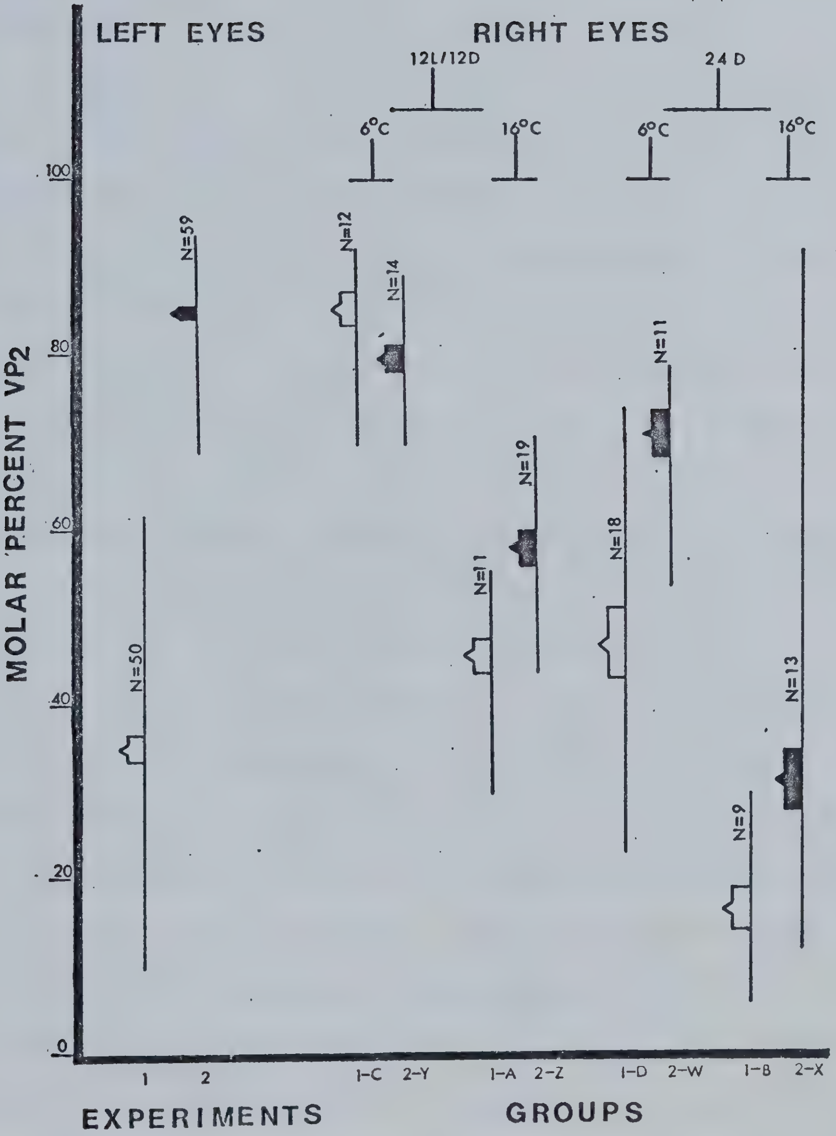
The results from Experiments 1 and 2 are summarized in Figure 2. Fish in Experiment 1 had a low molar percent $VP_2$ (34.9%, n = 50) at the beginning of the experiment. At the end, the four groups of fish ranged from 85.2% in group 1-C to 16.4% in group 1-B. Under the two light regimes of 12L/12D and 24D, low temperature (6° C) in comparison to high temperature (16° C) favored higher molar percent $VP_2$ (compare groups 1-C to 1-A, 1-D to 1-B). Similarly, under the two temperature regimes of 6° C and 16° C, light (12L/12D) in comparison to darkness (24D) favored higher molar percent $VP_2$ (compare groups 1-C to 1-D and 1-A to 1-B). Light and low temperature (12L/12D, 6° C) induced the largest increase of molar percent $VP_2$ (group 1-C) whereas darkness and high temperature (24D, 16° C) induced the only decrease of molar percent $VP_2$ (group 1-B) amongst all four groups of fish. A similar magnitude of increase in molar percent $VP_2$ was found between groups 1-A (12L/12D, 16° C) and 1-D (24D, 6° C) suggesting that there was no significant interaction between the actions of light and temperature on visual pigment composition.

The molar percent $VP_2$ of fish in Experiment 2 was high (84.7%, n = 59) at the beginning of the experiment. At the end of 28 days, the molar percent $VP_2$ was found to range from 79.5% in group 2-Y to 31.2% in group 2-X. Under the two light regimes of 12L/12D and 24D, low

Figure 2. Visual pigment composition in fish at the beginning (left eyes) and at the end (right eyes) of Experiments 1 and 2 (groups 1-A, B, C, D, and groups 2-W, X, Y, Z).

Fish in Experiment 1 were preconditioned at 24D at 16° C for one month before the experiment began. Fish in Experiment 2 were subjected to experimental conditions immediately upon arrival from the rearing station. All fish were subjected to 28 days of the different light and temperature regimes indicated in the figure.

The vertical line represents the range, the vertical bar gives one standard error from the mean, N = sample size. Results from Experiment 1 are represented by white bars and Experiment 2, black bars.


LEFT EYES
RIGHT EYES
12L/12D
24 D
6°C
16°C
6°C
16°C
MOLAR PERCENT VP2
100
80
60
40
20
0
N=50
N=59
N=12
N=14
N=11
N=19
N=18
N=11
N=9
N=13
1
2
1-C
2-Y
1-A
2-Z
1-D
2-W
1-B
2-X
EXPERIMENTS
GROUPS

temperature (6° C) in comparison to high temperature (16° C), favored higher molar percent $VP_2$ (compare groups 2-Y to 2-Z and 2-W to 2-X). Similarly, under the two temperature regimes of 6° C and 16° C, light (12L/12D) in comparison to darkness (24D) favored higher molar percent $VP_2$ (compare groups 2-Y to 2-W and 2-Z to 2-X). Light and low temperature (12L/12D, 6° C) induced the least amount of decrease of molar percent $VP_2$ (group 2-Y) whereas darkness and high temperature (24D, 16° C) induced the largest decrease of molar percent $VP_2$ (group 2-X) amongst all four groups of fish. Darkness and low temperature (24D, 6° C) favored a higher molar percent $VP_2$ (group 2-W) than light and high temperature (12L/12D, 16° C, group 2-Z). However, Student's T test shows that the molar percent $VP_2$ of the two groups (2-W and 2-Z) of fish are not significantly different from each other ($p < 0.05$).

Results from factorial analysis of variance (Sokal and Rohlf, 1969) on molar percent $VP_2$ of right eyes of all fish in Experiments 1 and 2 show that light and temperature effects are statistically significant in both experiments (Table 1; $p \leq 0.05$ is hereon designated as accepted level for significance). This indicated that in both experiments, under the two temperature regimes of 6° C and 16° C, light (12L/12D) in comparison to darkness (24D) favored significantly higher molar percent $VP_2$. Under the two light regimes of 12L/12D and 24D, low temperature (6° C) in comparison to high temperature (16° C) also favored significantly higher molar percent $VP_2$. For the four different combinations of light and temperature regimes studied, light and low temperature (12L/12D, 6° C) favored the highest molar percent $VP_2$ whereas darkness and high temperature (24D, 16° C) favored lowest molar percent $VP_2$. These phenomena occurred apparently irrespective of whether the

Table 1. Results of factorial analysis of variance on data from Experiments 1 through 6

| | Variables analysed | Factor* | Result** |
|---|---|---|---|
| Molar percent $VP_2$ of right eyes at end of experiment | Experiment 1 | A<br>B<br>AxB | S<br>S<br>NS |
| | Experiment 2 | A<br>B<br>AxB | S<br>S<br>NS |
| | Experiment 5 | A<br>B<br>AxB | S<br>S<br>S |
| | Experiment 6 | A<br>C<br>AxC | S<br>S<br>NS |
| Thyroxine levels in blood at end of experiment | Experiment 1 | A<br>B<br>AxB | NS<br>NS<br>S |
| | Experiment 2 | A<br>B<br>AxB | NS<br>NS<br>NS |
| | Experiment 5 | A<br>B<br>AxB | NS<br>S<br>S |
| | Experiment 6 | A<br>C<br>AxC | NS<br>S<br>NS |
| Percent 3-dehydroretinol in blood at end of experiment | Experiment 5 | A<br>B<br>AxB | S<br>S<br>NS |
| | Experiment 6 | A<br>C<br>AxC | NS<br>S<br>NS |

*A: Light effect
B: Temperature effect
C: Thyroxine introduction to tank water
x: Interaction between 2 factors.

**S = significance; NS = non-significance; significance is recognised at the 5% level.

molar percent $VP_2$ was high or low at the beginning of the experiments.

The change of visual pigment composition over time was studied by serially sampling fish every three days for a period of 45 days (Experiments 3 and 4). Results from Experiment 3 indicated again that at high temperature (16° C), light (24L or 12L/12D) in comparison to darkness (24D) favored higher molar percent $VP_2$ (Fig. 3). The magnitude of the decrease of molar percent $VP_2$ among groups 3-M, 3-L, and 3-N did not seem to be proportional to the decrease of photoperiod in each case (24L, 12L/12D and 24D). Also the change of visual pigment composition did not appear to have reached any equilibrium level (where there was minimal change of visual pigment composition over time) within the 45 days of the experiment.

Results from Experiment 4 (Fig. 4) showed that in total darkness (24D), low temperature (5° C) in comparison to high temperature (15° C) favored higher molar percent $VP_2$. The magnitude of the decrease of molar percent $VP_2$ among groups 4-T, 4-U and 4-K appeared to be proportional to the increase of temperature in each case (5° C, 10° C and 15° C). In addition, the estimated lines representing the change of visual pigment composition seemed to indicate that the fish had reached (or moved close to) new equilibria of visual pigment composition by the 30th day of experiment.

The unilateral effect of light on eyes of fish with black and transparent caps was studied in Experiment 7 (Table 2). Results from eight fish kept under the experimental condition (24L, 8° C) for a period of 10 days showed a mean difference of 20.7% between the two eyes fitted with black and transparent caps (see Table 2). Therefore the unilateral

Figure 3. Effect of light on the visual pigment composition in fish over a period of 45 days (Experiment 3).

Experiment began upon arrival of fish from the rearing station. Left eyes of fish were removed at the beginning of the experiment (white column at day zero, shaded portion of which shows one standard error from the mean). Right eyes from two fish per group, whenever possible, were removed every three days during the experiment. The fish were held at 16° C under 24L (group 3-M, white columns, connected by broken line), 12L/12D (group 3-L, shaded columns, connected by solid line), and 24D (group 3-N, black columns, connected by broken and dotted line). The lines were fitted by eye estimation.

Upper and lower ends of vertical column show range of sample. Horizontal line shows the mean. Sample size is two per column unless otherwise indicated. Only one result (●) was obtained on the sixth days of experiment in group 3-L (12L/12D).

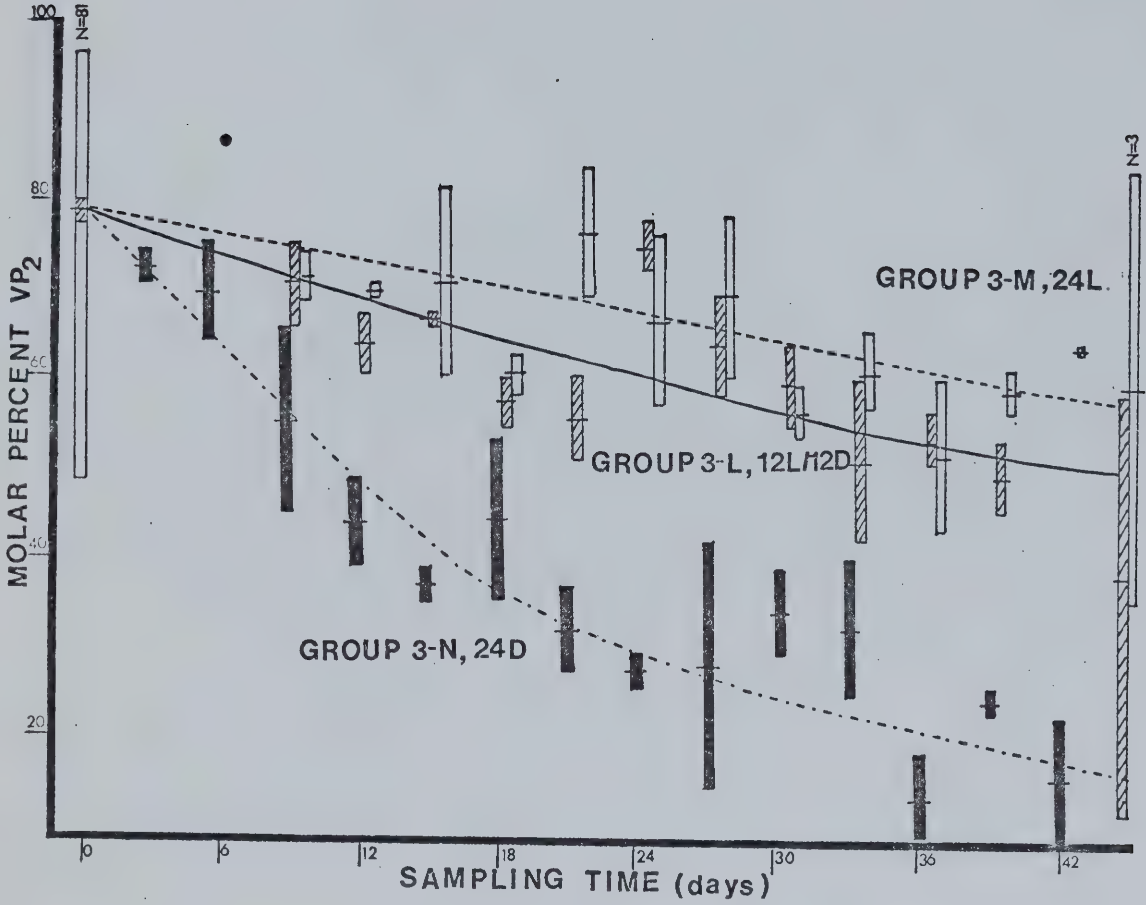

N=81
N=3
100
80
60
40
20
MOLAR PERCENT VP$_2$
GROUP 3-M, 24L
GROUP 3-L, 12L/12D
GROUP 3-N, 24D
0
6
12
18
24
30
36
42
SAMPLING TIME (days)

Figure 4. Effect of temperature on the visual pigment composition over a period of 45 days (Experiment 4).

Fish were preconditioned at 4-5° C under laboratory condition for approximately one month before the experiment began, when left eyes of the fish were removed (white column at day zero, shaded portion of which shows one standard error from the mean). Right eyes from two fish per group, whenever possible, were removed every three days during the experiment. The fish were held in total darkness at 5° C (group 4-T, white columns, connected by broken line), 10° C (group 4-U, shaded columns, connected by solid line) and 15° C (group 4-K, black columns, connected by broken and dotted line). The lines were fitted by eye estimation.

Upper and lower ends of vertical columns show range of sample. Horizontal line shows the mean. Sample size is two per column unless otherwise indicated. In group 4-T, only one result (●) was obtained on each of the 20th, 22nd, 23rd, and 42nd days of experiment.

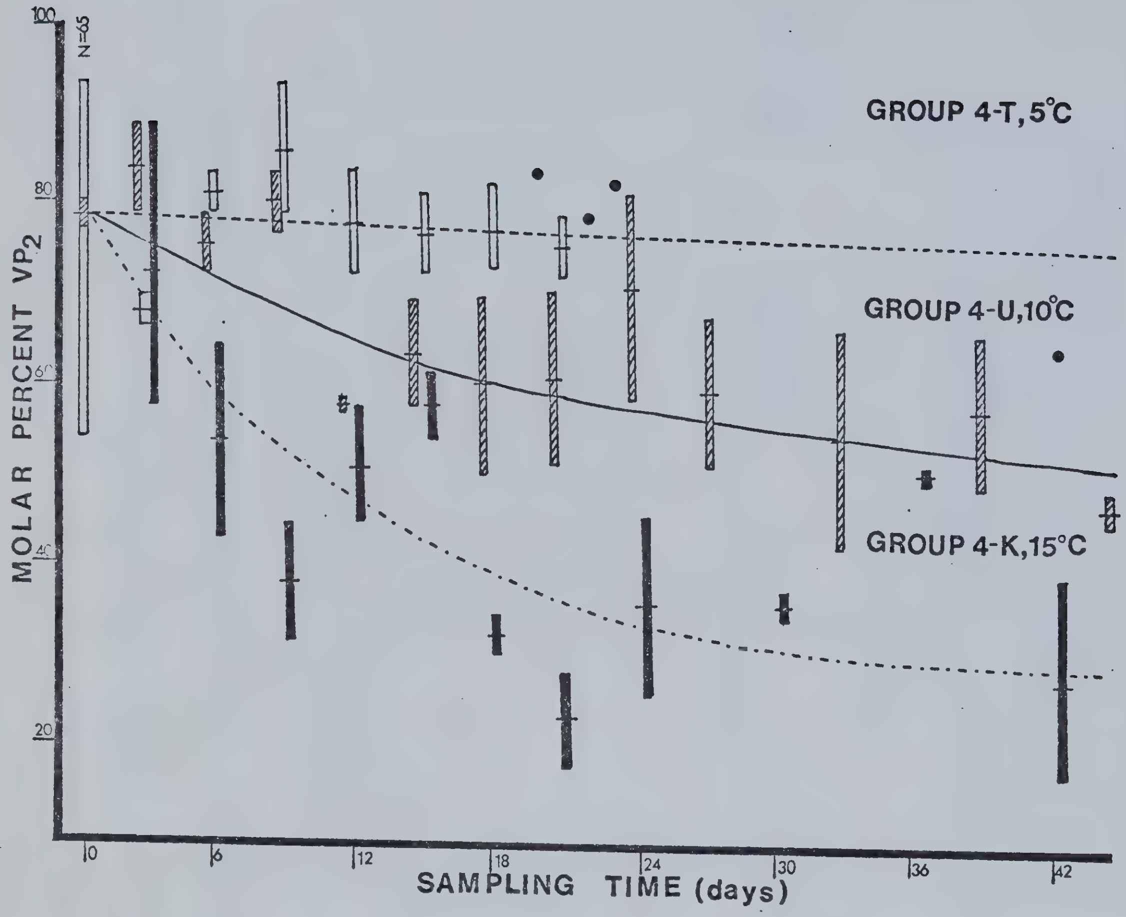

N=65
GROUP 4-T, 5°C
GROUP 4-U, 10°C
GROUP 4-K, 15°C
MOLAR PERCENT $VP_2$
100
80
60
40
20
0
6
12
18
24
30
36
42
SAMPLING TIME (days)

Table 2. Difference of molar percent $VP_2$ between two eyes of fish fitted with black and transparent eye caps. Fish in Experiment 7 were preconditioned at 16° C in total darkness for approximately three weeks before they were individually fitted with various combinations of black and transparent eye caps, as indicated in the table. During the experiment, they were held at 8° C under continuous illumination (24L). For values of molar percent $VP_2$ in each fish, see Table 23 in Appendix 7.

| No. of days caps successfully stayed on fish eyes | Description of capping conditions* | Sample size | Difference of molar percent $VP_2$ of two eyes |
|---|---|---|---|
| 3 | A | 1 | 7.9%** |
| 4 | A | 2 | 10.5%** |
| 5 | A | 1 | 9.2%** |
| 10 | A | 8 | 20.7%** |
| 6 | B | 1 | 2.5%*** |
| 4 | C | 1 | 4.6%**** |
| 10 | C | 1 | 0.0%**** |

*A: Fish fitted with one black and one transparent cap.
B: Fish fitted with transparent caps on both eyes.
C: Fish fitted with black caps on both eyes.

**Molar percent $VP_2$ from eye fitted with transparent cap - molar percent $VP_2$ from eye fitted with black caps.

***Molar percent $VP_2$ from right eye - molar percent $VP_2$ from left eye.

****Molar percent $VP_2$ from left eye - molar percent $VP_2$ from right eye.

action of light on changes of visual pigment composition in two eyes of the same fish was evident. Fish fitted with both black caps and both transparent caps showed differences of molar percent $VP_2$ less than 5% between the two eyes, irrespective of the number of days the caps successfully stayed on the eyes of the fish.

## II. EFFECT OF EXOGENOUS THYROXINE AND INFLUENCE OF PHYSIOLOGICAL LEVEL OF THYROXINE ON VISUAL PIGMENT COMPOSITION

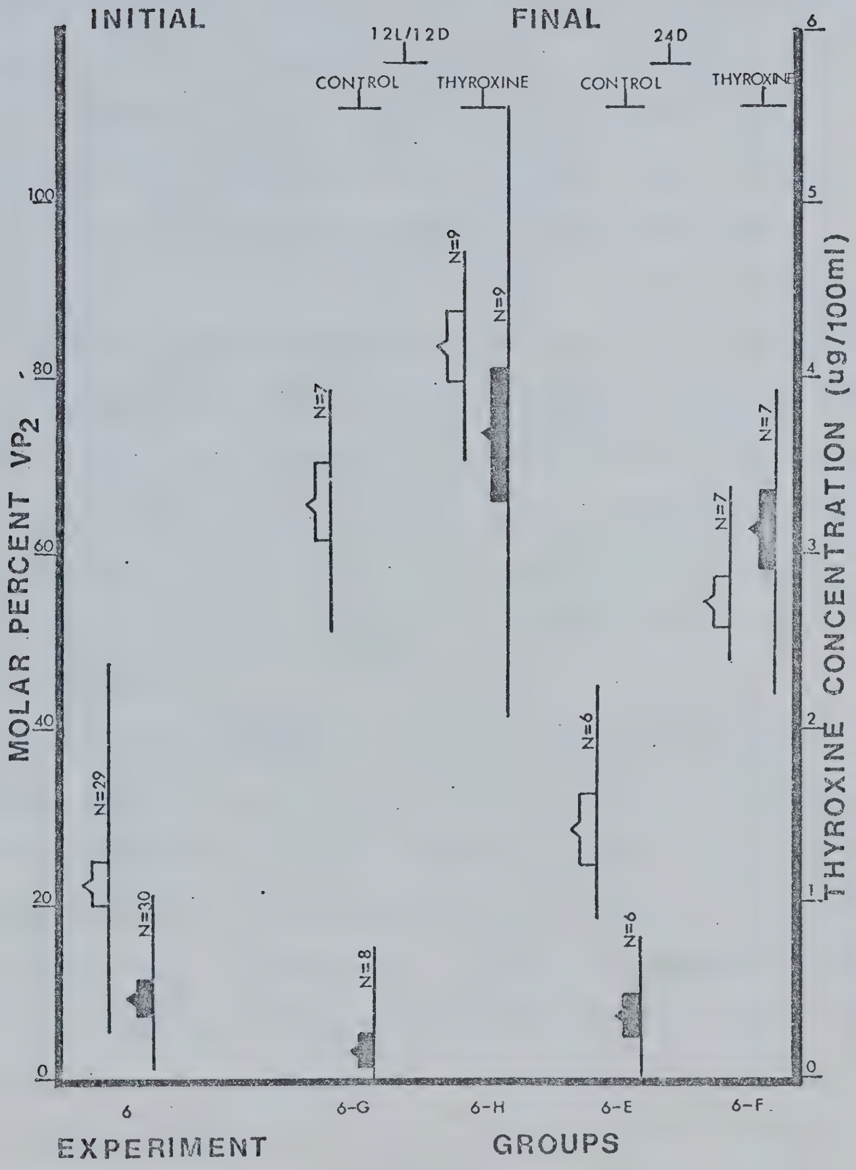
The effect of exogenous thyroxine on visual pigment composition was studied in Experiment 6 (Fig. 5). Under the two light regimes of 12L/12D and 24D, introduction of L-thyroxine to tank water (10 μg L-thyroxine/100 ml tank water) induced significantly higher levels of both thyroxine concentration in blood and molar percent $VP_2$ in retina of the fish (compare groups 6-H to 6-G and 6-F to 6-E; see Table 1 for level of significance). Therefore, the action of exogenous thyroxine in elevation of molar percent $VP_2$ in rainbow trout is evident.

Comparison of the molar percent $VP_2$ between the two groups of thyroxine-treated fish (compare group 6-F to 6-H) or the two groups of controls (compare group 6-E to 6-G) shows that light (12L/12D) in comparison to darkness (24D) induced significantly higher molar percent $VP_2$ in the trout. However, light (12L/12D) in comparison to darkness (24D) did not induce any significant difference in the thyroxine levels between the two groups of thyroxine-treated fish (compare group 6-H to 6-F) or the two groups of controls (compare group 6-G to 6-E). An analysis of variance (Table 1) also indicates insignificant interaction

Figure 5. Visual pigment composition and thyroxine concentration in fish with thyroxine introduced into tank water at a concentration of 100 μg/100 ml (Experiment 6, groups 6-E, F, G and H).

Fish were preconditioned at 16° C, 24D for 30 days before the beginning of the experiment when left eyes and blood samples were obtained from the fish. All fish were held at 8° C for 10 days in experimental conditions indicated in the Figure. Right eyes and blood samples were again obtained from the fish at the end of the experiment.

The vertical line represents the range, the vertical bar gives one standard error from the mean, N = sample size. Thyroxine concentrations in blood are represented by black bars and molar percent $VP_2$ by white bars.


INITIAL
FINAL
12L/12D
24D
CONTROL
THYROXINE
CONTROL
THYROXINE
MOLAR PERCENT VP$_2$
THYROXINE CONCENTRATION (ug/100ml)
0
20
40
60
80
100
0
1
2
3
4
5
6
N=29
N=30
N=7
N=8
N=9
N=9
N=6
N=6
N=7
N=7
6
6-G
6-H
6-E
6-F
EXPERIMENT
GROUPS

effects between exogenous thyroxine and light as factors affecting visual pigment composition in the fish.

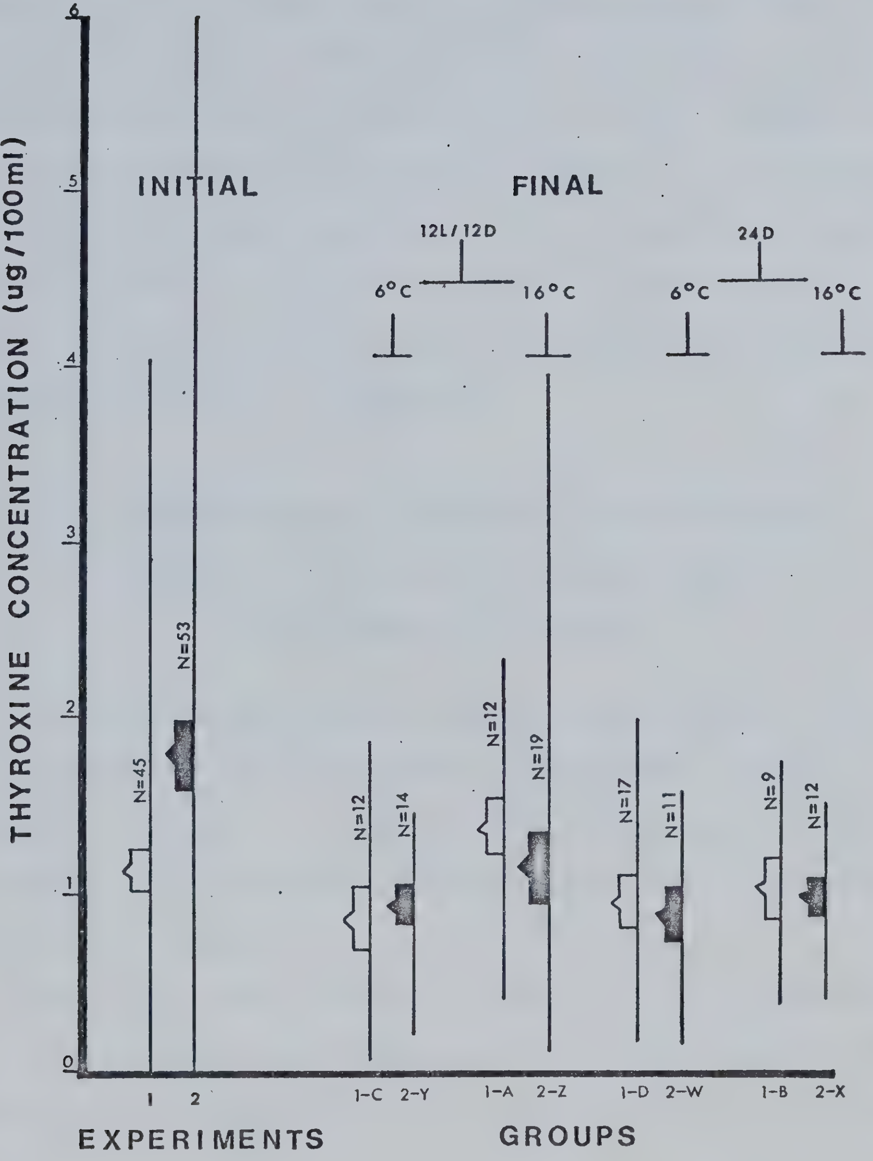
In the previously described Experiments 1 and 2, levels of thyroxine concentration in blood, in addition to molar percent $VP_2$ were determined in fish at the beginning and end of the experiments (Fig. 6). Under the two different light regimes of 12L/12D and 24D, high temperature (16° C) in comparison to low temperature (6° C) favored slightly higher levels of thyroxine in blood in both experiments (compare group 1-A to 1-C, 1-B to 1-D, 2-Z to 2-Y and 2-X to 2-W). Under the two different temperature regimes of 16° C and 6° C, light (12L/12D) in comparison to darkness (24D), favored also slightly higher levels of thyroxine in three cases (compare group 1-A to 1-B, 2-Y to 2-W and 2-Z to 2-X) but lower levels in one (compare group 1-C to 1-D). Factorial analysis of variance (Table 1) shows that both light and temperature effects on the blood level of thyroxine are statistically non-significant in these experiments.

Jacquest (1969) working with rainbow trout and Allen (1971) working with redside shiner reported that bringing fish from outdoor to laboratory conditions resulted in a decrease of percent $VP_2$. In the experiments carried out in this study, the same effect was noted (Fig. 2, Experiment 2; Fig. 3, Experiment 3; and Fig. 7, Experiment 5). In Experiment 2, thyroxine levels were determined from blood samples taken from fish within one or two days upon arrival from the rearing station. The mean value was found to be comparatively high (1.79 µg/100 ml, $n = 53$, see Appendix 7 for mean values in other groups). When fish in this experiment were resampled after 28 days, a decrease of molar percent $VP_2$

Figure 6. Thyroxine concentration in blood samples from fish in Experiments 1 and 2 (groups 1-A, B, C, D and 2-W, X, Y, Z).

Fish in Experiment 1 were preconditioned at 24D at 16° C for one month before the experiment began. Fish in Experiment 2 were subjected to experimental conditions immediately upon arrival from the rearing station. All fish were subjected to 28 days of the different light and temperature regimes indicated in the Figure.

The vertical line represents the range, the vertical bar gives one standard error from the mean, N = sample size. Results from Experiment 1 are represented by white bars and Experiment 2, black bars.


THYROXINE CONCENTRATION (ug/100ml)
0
1
2
3
4
5
6
INITIAL
FINAL
12L/12D
24D
6°C
16°C
6°C
16°C
N=45
N=53
N=12
N=14
N=12
N=19
N=17
N=11
N=9
N=12
1
2
1-C
2-Y
1-A
2-Z
1-D
2-W
1-B
2-X
EXPERIMENTS
GROUPS

in all groups was evident (Fig. 2). Comparing results (from the same fish) at the beginning and at the end of Experiment 2, there was a significant decrease of molar percent $VP_2$ associated with a significant decrease of thyroxine concentration in fish from groups 2-X (24D, 16° C) and 2-Z (12L/12D, 16° C). This association of higher thyroxine levels with higher molar percent $VP_2$ in outdoor fish and lower thyroxine levels and lower molar percent $VP_2$ in indoor fish suggests that there might exist some form of relationship between the two parameters, although correlation coefficients calculated from all individual groups using thyroxine levels and molar percent $VP_2$ of the same fish as pairs of independent variables were insignificant.

## III. RELATIONSHIP AMONG CIRCULATORY LEVEL OF THYROXINE, PERCENT 3-DEHYDRORETINOL IN BLOOD AND VISUAL PIGMENT COMPOSITION IN RETINA

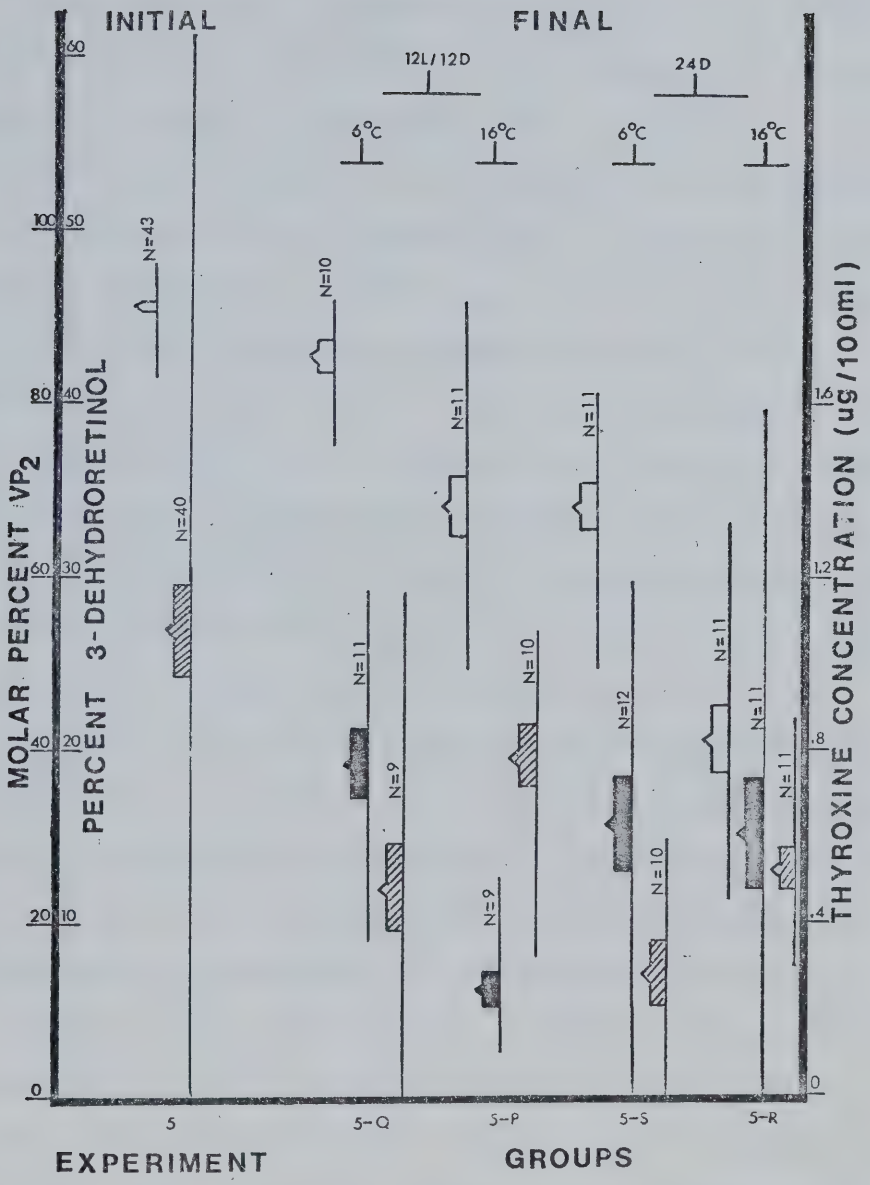
The percent 3-dehydroretinol in blood and molar percent $VP_2$ at the beginning and at the end of Experiment 5 are presented in Figure 7. Owing to the small amount of blood (0.6 ml) obtained from each fish at the beginning of the experiment, thyroxine levels were not determined from the initial samples.

Under the two light regimes of 12L/12D and 24D, high temperature (16° C) in comparison to low temperature (6° C) favored higher percent 3-dehydroretinol in blood and lower molar percent $VP_2$ in retina (compare group 5-P to 5-Q and 5-R to 5-S). Under the two temperature regimes of 6° C and 16° C, light (12L/12D) in comparison to darkness (24D) favored higher percent 3-dehydroretinol in blood and molar percent $VP_2$ in retina

Figure 7. Visual pigment composition, thyroxine concentration and percent 3-dehydroretinol in blood sampled from fish in Experiment 5 (groups 5-P, Q, R and S).

Experiment began upon arrival of fish from the rearing station. Left eyes of fish were removed at the beginning of the experiment. Blood samples obtained were analyzed for percent 3-dehydroretinol. After being subjected to the different experimental conditions (as indicated in the Figure) for 26 or 29 days, right eyes were removed from fish with the blood samples obtained.

The vertical line represents the range, the vertical bar gives one standard error from the mean, N = sample size. Thyroxine concentrations in blood are represented by black bars, percent 3-dehydroretinol by shaded bars and molar percent $VP_2$ by white bars.


INITIAL
FINAL
12L/12D
24D
6°C
16°C
6°C
16°C
MOLAR PERCENT VP2
PERCENT 3-DEHYDRORETINOL
THYROXINE CONCENTRATION (ug/100ml)
0
20
40
60
80
100
10
20
30
40
50
60
.4
.8
1.2
1.6
0
N=43
N=40
N=10
N=11
N=9
N=11
N=9
N=10
N=11
N=12
N=10
N=11
N=11
N=11
5
5-Q
5-P
5-S
5-R
EXPERIMENT
GROUPS

(compare group 5-Q to 5-S and 5-P to 5-R). Factorial analysis shows that both light and temperature effects on molar percent $VP_2$ and percent 3-dehydroretinol are significant (Table 1).

Results on levels of thyroxine are inconclusive. (1) At 12L/12D, low temperature (6° C) in comparison to high temperature (16° C) favored significantly (Student's T test) higher levels of thyroxine concentration (compare group 5-Q to 5-P) whereas at 24D, low temperature (6° C) in comparison to high temperature (16° C) favored only a slightly higher level of thyroxine in blood (compare group 5-S to 5-R). However, in the two previously described experiments (Experiments 1 and 2, Figure 6) high temperature (16° C) in comparison to low temperature (6° C) favored slightly higher levels of thyroxine under the two light regimes of 12L/12D and 24D. (2) Under the two different temperature regimes of 16° C and 6° C, light (12L/12D) in comparison to darkness (24D) favored higher thyroxine levels in one case (at 6° C, compare group 5-Q to 5-S) and lower levels in another (at 16° C, compare group 5-P to 5-R).

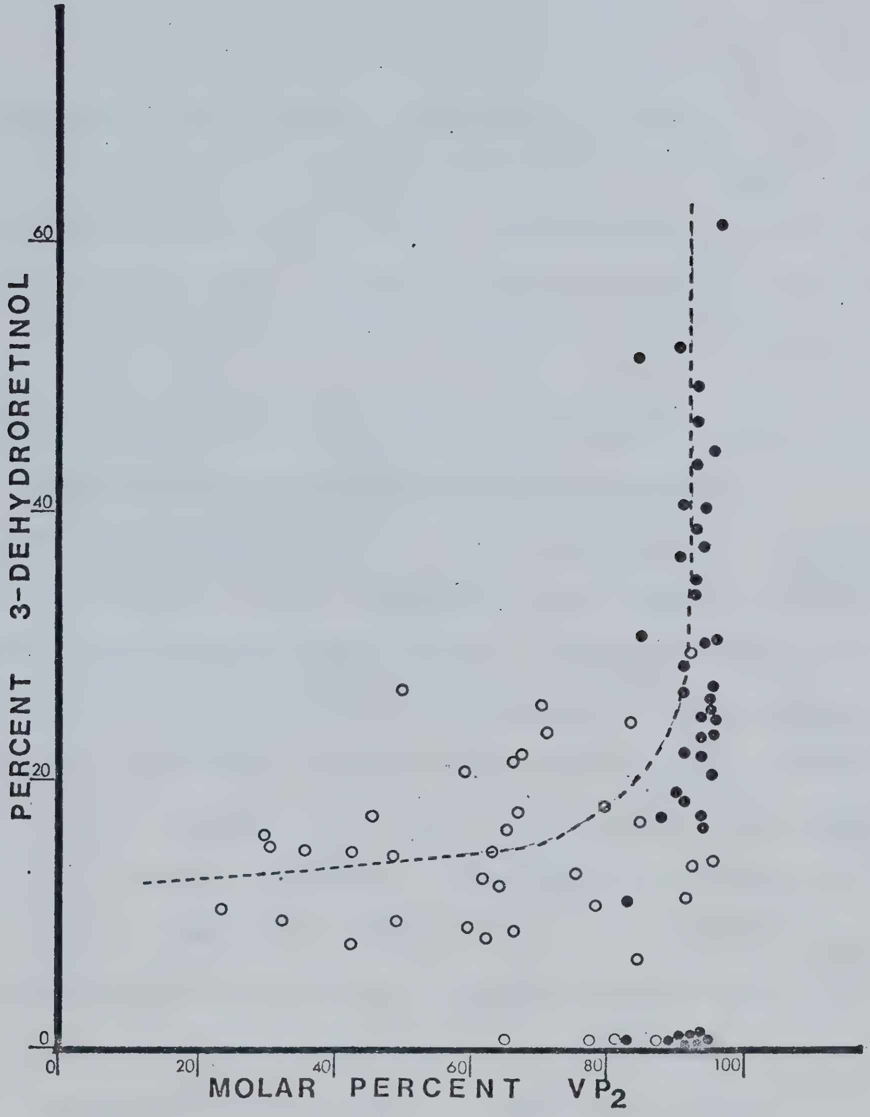
No significant correlations were found between thyroxine levels with molar percent $VP_2$, molar percent $VP_2$ with percent 3-dehydroretinol and percent 3-dehydroretinol with thyroxine. Inspection of Figure 8 shows that decrease of percent 3-dehydroretinol in all groups during the experiment seems to be associated with the decrease of molar percent $VP_2$. Student's T tests show that percent 3-dehydroretinol decreased significantly in group 5-P, associated with a significant decrease of molar percent $VP_2$ in the same group. Therefore, one can speculate that there may exist some relationship of higher percent 3-dehydroretinol in blood with higher molar percent $VP_2$ in the retina.

Factorial analysis of variance shows that introduction of thyroxine

Figure 8. The relation of visual pigment composition to the percent 3-dehydroretinol in blood (as sampled at the beginning and end of Experiment 5).

Experiment began upon arrival of fish from the rearing station. Left eyes of fish were removed at the beginning of the experiment. Blood samples obtained were analyzed for percent 3-dehydroretinol. After being subjected to the different experimental conditions (12L/12D at 6° C and 16° C; 24D at 6° C and 16° C) for 26 to 29 days, right eyes were removed from fish with the blood samples obtained.

Each black dot represents result from one fish sampled at the beginning of the experiment and each white dot represents result from one fish sampled at the end of the experiment.


PERCENT 3-DEHYDRORETINOL
60
40
20
0
0
20
40
60
80
100
MOLAR PERCENT $VP_2$

to tank water significantly (Table 1) increased percent 3-dehydroretinol in blood (Table 3). However, owing to the small sample size in each group, this finding requires further substantiation.

## IV. OTHER RELATIONSHIPS

### A. Body Weight Versus Thyroxine Concentration in Blood

Some 300 determinations showed that smaller fish tended to have higher blood thyroxine levels (Fig. 9). Higgs and Eales (1973), using the same Tetralute method, reported an even lower mean value of 0.39 μg/ 100 ml from seven rainbow trout with a mean body weight of 343 g.

### B. Change in Optical Density Unit ($^{\Delta}$D max: Amount of Extracted Pigment) in Relation to Visual Pigment Composition

There had been contradictory results reported on the relationship between total amount of visual pigment and visual pigment composition in the retina. Bridges (1965) reported that in *Belonesox*, *Notemigonus* and *Scardinius*, changes in visual pigment composition did not accompany a change in the total amount of visual pigment recovered by extraction from the retina. However, Allen (1971) showed that the total amount of visual pigment in retina increases as molar percent $VP_2$ increases. Results from all experiments in the study were used to determine the amount of visual pigment in relation to molar percent $VP_2$ (Fig. 10). A regression formula was calculated (based on the mean values in each group) to describe the best-fit line. A significant decrease in the amount of visual pigment in the retina is found to be associated with an increase of molar percent $VP_2$. This finding does not agree with those reported by Allen (1971) and Bridges (1965).

Table 3. Mean values of percent 3-dehydroretinol and thyroxine concentration in blood at the end of Experiment 6. Fish were preconditioned at 16° C, 24D for 30 days before the beginning of the experiment. The left eyes and blood samples were obtained from the fish. All fish were held at 8° C for 10 days in experimental conditions indicated in the table. Right eyes and blood samples were again obtained from the fish at the end of the experiment.

| Groups | 6-E<br>24D<br>Control | 6-F<br>24D<br>Thyroxine* | 6-G<br>12L/12D<br>Control | 6-H<br>12L/12D<br>Thyroxine* |
|---|---|---|---|---|
| Thyroxine concentration in blood (μg/100 ml) | 0.35<br>(n=6) | 3.10<br>(n=7) | 0.16<br>(n=8) | 3.70<br>(n=9) |
| Percent 3-dehydroretinol in blood | 4.3<br>(n=6) | 6.8<br>(n=7) | 4.5<br>(n=4) | 16.3<br>(n=6) |

*L-thyroxine was introduced to tank water at a concentration of 10 μg/100 ml.

Figure 9. The relation of thyroxine concentration in blood to the mean body weight of fish (as sampled at the beginning and at the end of Experiments 1, 2, 5 and 6; exclusive of the results from the thyroxine-treated fish).

(For experimental protocols, see Materials and Methods.)

Black dot represents the mean of the group sampled at the beginning of the experiment; white dot, the end. Vertical line shows one standard error from the mean. Sample size in each group ranges from 6 to 19. Results from 299 determinations are presented in the Figure.

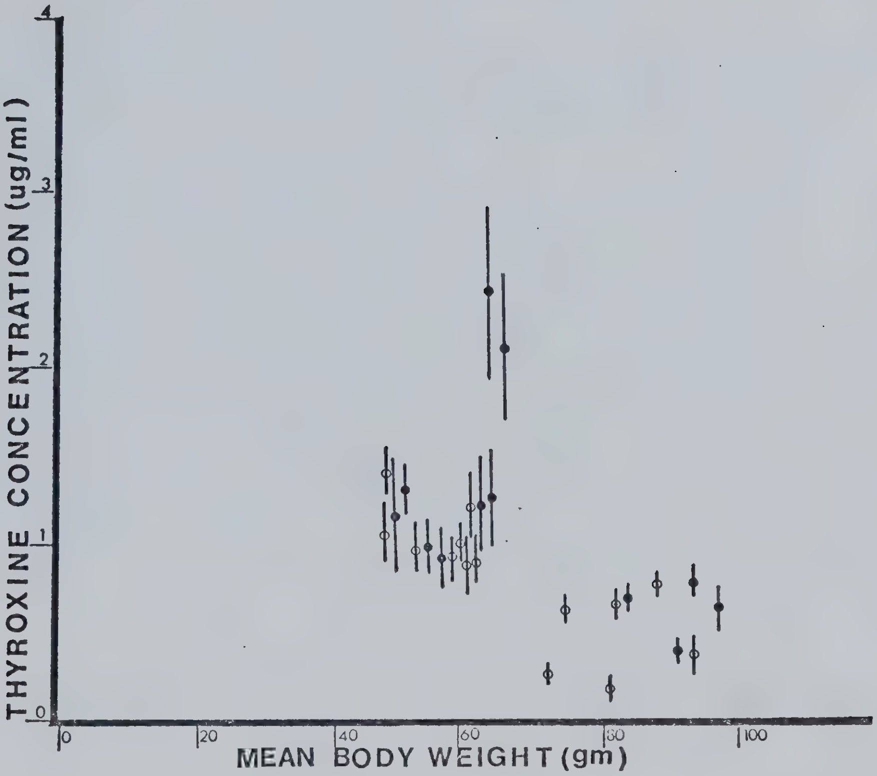
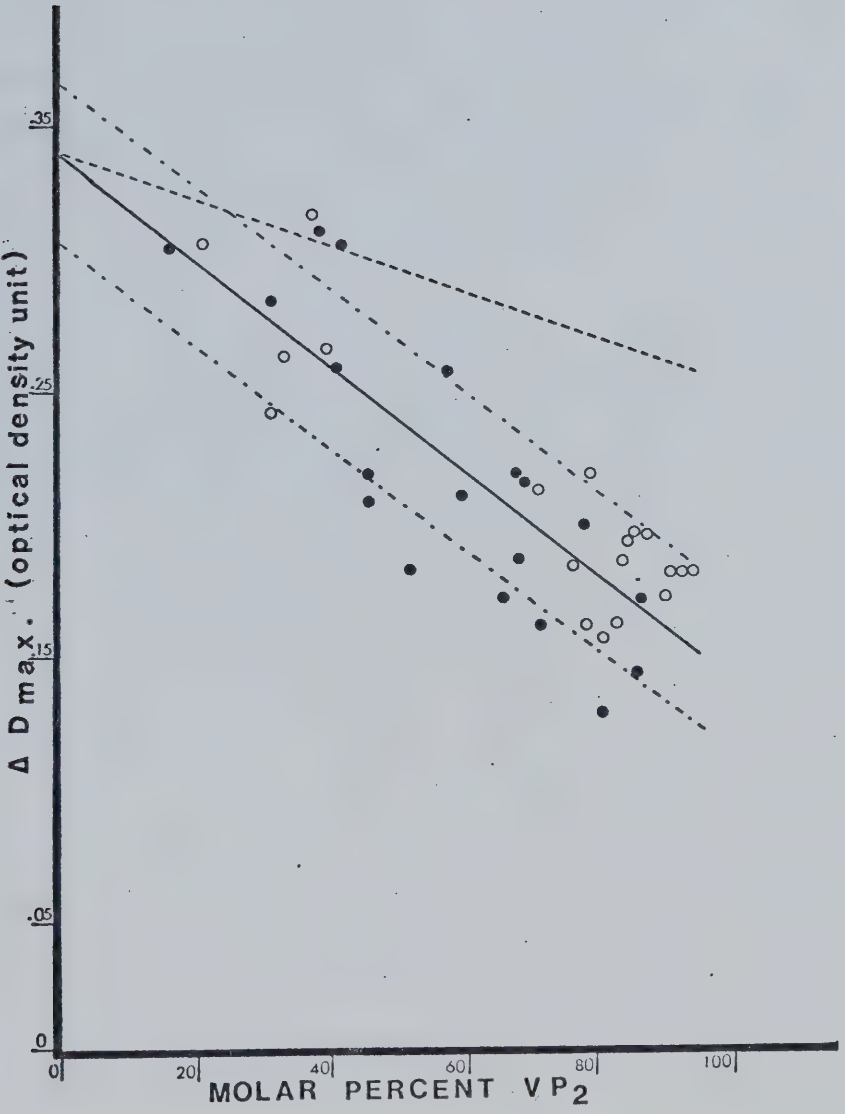

THYROXINE CONCENTRATION (ug/ml)
0
1
2
3
4
0
20
40
60
80
100
MEAN BODY WEIGHT (gm)

Figure 10. The relation of visual pigment composition to the amount of extracted visual pigment ($\Delta D_{max}$) in the retina. Values come from results from Experiments 1 to 7.

Mean values for each group (with sample size ranges from 9 to 29 per group) are shown. Results from left eyes (beginning of experiments) are represented by white dots and right eyes (end of experiments) by black dots. Regression formula calculated from the mean values in each group is: $Y = 0.3397 - 0.002X$ (solid line), $t = 11$, $p < 0.001$ (significantly different from zero slope). Broken and dotted lines show 95% confidence limit of the regression line. Broken line shows expected change of optical density unit along the molar percent $VP_2$ scale owing to the difference of molar extinction coefficients of rhodopsin ($E_{max} = 40{,}600$) and porphyropsin ($E_{max} = 30{,}000$) (Dartnall, 1968). When the slope of this line (0.008) is compared to that of the regression line, result also shows significance ($t = 5.5$, $p<0.001$).


.35
.25
.15
.05
0
Δ D max. (optical density unit)
0
20
40
60
80
100
MOLAR PERCENT VP$_2$

### C. Effect of Bleeding on Thyroxine Concentration in Blood and Visual Pigment Composition in Retina

At the beginning of Experiments 1 and 2, an average of 0.3 ml of blood was taken from each fish except in ten, which were not bled (5 in group 1-D and group 2-Z). Assuming the circulatory levels of thyroxine and visual pigment composition of these ten fish did not differ significantly from that of the population from which they were randomly selected, the results obtained from these fish at the end of the experiment can then be compared to the rest of the fish in the same group for the effect of bleeding. Student's T tests were employed for this purpose. Both circulatory levels of thyroxine and molar percent $VP_2$ in these fish did not differ significantly from values in other fish in the same groups (see Appendix 7, Tables 4 and 8).

# DISCUSSION

This study has provided information on the effect of several important environmental (light, temperature) and physiological (thyroxine level and percent 3-dehydroretinol in blood) factors on visual pigment composition in juvenile rainbow trout (*Salmo gairdneri*).

## ROLE OF LIGHT

In this study, contradictory reports on the effect of light and darkness on the visual pigment composition of rainbow trout (see Introduction) are partially resolved. Results from this study agree with those reported by Allen *et al.* (1973) that light in comparison to darkness favored higher percent $VP_2$. Studies on the change of visual pigment composition over time (Fig. 3, Experiment 2) did not provide sufficient information to render any possible extrapolation of indoor to outdoor situations.

Results from the capped-eye experiment (Table 2) suggested the possible existence of a local control mechanism for the effect of light on visual pigment composition. The exposure of ocular tissue to a light-dark cycle in comparison to continuous darkness increases the rate of bleaching and subsequent resynthesis of visual pigment, thereby increasing the rate of turnover (degradation and synthesis) of visual pigments and their prosthetic groups in the receptor cells (Bridges and Yoshikami, 1970; Hall and Bok, 1974). In addition, Bridges and Yoshikami

(1970c) suggested that light might influence the 3-4 dehydrogenation reaction (which converts retinol to 3-dehydroretinol), hypothesized to take place in the "light sensitive" myeloid body (Yamada, 1961) in the cells of the pigment epithelium. Recent information also indicates that some enzymatic reactions (such as kinase activity) in the retinal outer segment are light-sensitive (Bownds, *et al.*, 1974; Frank and Bensinger, 1974).

## ROLE OF TEMPERATURE

The study showed that low temperature (5-6° C) in comparison to high temperature (15-16° C) favored higher molar percent $VP_2$. This is in accordance with the results reported by Allen and McFarland (1973) who worked on the golden shiner, *Notemigonus crysoleucas*. Note that the latter is a cyprinid fish which responds to light by decreasing the proportion of porphyropsin (Bridges, 1974; Allen and McFarland, 1973). Therefore, it is evident that temperature exerts a similar effect on fish which respond to light by increasing (rainbow trout) or decreasing (golden shiner) the percent porphyropsin. In spite of the opposite effects of light and darkness on visual pigment composition in different fishes, there exists a general tendency towards increasing the proportion of porphyropsin in the winter and decreasing it in the summer (Allen and McFarland, 1973, Table 5, p. 911). Therefore the importance of temperature, as an environmental factor, on visual pigment composition in fishes should not be overlooked.

In total darkness, new equilibria of visual pigment composition were reached within thirty days after the fish were transferred to

different temperatures (Fig. 4, Experiment 4). However, there was no indication that equilibria were established during the 45-day period when fish were held under 24L, 12L/12D or 24D at high temperature (16° C) (Fig. 3, Experiment 3). Certain contradictory reports on the effect of light on visual pigment composition in certain salmonid fishes (Beatty, 1966, juvenile coho salmon and king salmon, *Oncorhynchus tshawytscha*) might be explained by the temperature effect (Beatty, 1975, personal communication).

The mechanism whereby temperature might exert its influence on visual pigment conversion can be summarized as follows:

1) Temperature could affect the bleaching-resynthesis cycle thereby controlling the rate of turnover of visual pigment and their prosthetic groups in the receptor cells (Bridges, 1972).

2) Temperature might affect general metabolism of the receptor cells. For example, the rate of formation and subsequent displacement of outer segment discs in frog photoreceptors (rods) is doubled with a temperature increase of 10° C (Young, 1967).

3) The hypothesized 3-dehydrogenase enzyme (Dartnall, 1964; Bridges, 1965) responsible for conversion of retinol to 3-dehydroretinol might have optimal activity at high or low temperatures. Other fish dehydrogenases have been shown to have maximum activity at given temperatures (Hochachka and Somero, 1973).

The reason why low temperature favors porphyropsin may be associated with the thermal stability of rhodopsin and porphyropsin. Bridges (1956) found that rainbow trout porphyropsin decayed 40 times as fast as rhodopsin in an extract (at 3° C) containing the two pigments.

Schwanzara (1967) reported that porphyropsin was less common than rhodopsin in tropical fish. This may be related to the possible decreased thermal stability of porphyropsin at higher temperatures (Bridges, 1972).

## ROLE OF THYROXINE

This study provides the only report on the elevation of circulatory thyroxine in fish accompanied by an increase of molar percent $VP_2$ when exogenous thyroxine is introduced into tank water. The increase of molar percent $VP_2$ in the control fish during the ten days of the experiment was partially due to a temperature effect (see Materials and Methods, Experiment 6). However, compared to the control fish held at the same temperature, fish with thyroxine introduced in the tank water had significantly higher molar percent $VP_2$. Therefore the action of exogenous thyroxine in the induction of increased molar percent $VP_2$ was evident. The fact that thyroxine levels in the blood increased in association with increasing molar percent $VP_2$ suggests a possible central control mechanism. The finding that thyroxine elevates the proportion of porphyropsin in rainbow trout agrees with results reported by Munz and Swanson (1965) and Jacquest and Beatty (1972).

No conclusive evidence is evident from the results concerning the effect of light and temperature on the circulatory level of thyroxine. Although a significant temperature effect on thyroxine concentration in blood was noted in the results in Experiment 5 (Fig. 7), results from other experiments (Experiments 1 and 2, Fig. 6) did not seem to substantiate the finding. In addition, results from Experiment 6 (Fig. 5) showed that, in the thyroxine-treated fish, those held in light (12L/12D)

had significantly higher molar percent $VP_2$ than those held in darkness (24D) even though there was no significant difference in the elevated thyroxine levels in the blood between the two groups. These suggest that light in comparison to darkness influences visual pigment in trout probably through some other means than the induction of an elevated level of thyroxine in circulation. The finding that there was no interaction (effect) between light and exogenous thyroxine as factors significantly affecting visual pigment composition in trout (see Table 1) partially substantiated this view.

The mechanism whereby thyroxine acts has been a subject of speculation (Wilt, 1959; Naito and Wilt, 1962; Ohtsu *et al.*, 1964; Beatty, 1969, 1972; Jacquest and Beatty, 1972; Allen, 1971; Bridges, 1972). Thyroxine might influence the activity of the hypothesized 3-dehydrogenase enzyme which is responsible for conversion of retinol to 3-dehydroretinol (Wilt, 1959; Naito and Wilt, 1962; Ohtsu *et al.*, 1964). It might also be responsible for the availability of retinol and 3-dehydroretinol by regulating their absorption, metabolism, storage and/or transport (as suggested by Beatty, 1972; Jacquest and Beatty, 1972). Until further detailed investigations are undertaken along this line, no further discussion on this aspect can be presented.

## ROLE OF 3-DEHYDRORETINOL

Results from Experiments 5 and 6 show that the percent 3-dehydroretinol in fish were generally low, with mean values ranging from 42.6% to 4.3% (see Appendix 7). This is in agreement with results reported by Plack and Woodhead (1966) who noticed that approximately 15%

3-dehydroretinol always co-exists with circulatory retinol in the cod, *Gadus morhua* (which is a fish having only vitamin $A_1$ based pigment in the retina; Lythgoe, 1972). Comparing the percent 3-dehydroretinol in blood to the molar percent $VP_2$ in a retina of the same fish, one can conclude that the former does not mirror the molar percent $VP_2$ (Fig. 8). Since the percent 3-dehydroretinol in the pigment epithelium mirrors percent $VP_2$ in fish (Wald, 1939; Bridges and Yoshikami, 1970b), it seems evident that the ocular tissue plays the more important role in determining the percent 3-dehydroretinol in the pigment epithelium rather than the blood level of the vitamins $A_1$ and $A_2$.

Beatty (1972) and Jacquest and Beatty (1972), working on rainbow trout and kokanee, found that either by intraperitoneal injections or through dietary intake of 3-dehydroretinol, significant increases of percent $VP_2$ occurred in the two species. This suggests that an increase of the percent 3-dehydroretinol in blood would probably increase the proportion of porphyropsin. In this study, high temperature (16° C), in comparison to low temperature (6° C) and light (12L/12D), in comparison to darkness (24D), favored higher percent 3-dehydroretinol in blood (Fig. 7). Since higher temperature also favors lower molar percent $VP_2$, it would not be likely that temperature, as an environmental factor, influences visual pigment composition via regulation of percent 3-dehydroretinol in blood. On the other hand, light in comparison to darkness might exert its influence on visual pigment composition by inducing a small increase of percent 3-dehydroretinol in blood.

Introduction of thyroxine to tank water has been shown to significantly increase percent 3-dehydroretinol in blood (Table 1). Owing to the large variance within each group and small sample size, this

evidence requires further substantiation (Table 3).

No experimental evidence has yet been reported favoring the hypothesis that the ocular tissue has the ability to differentially sequester the two vitamins from circulation thereby regulating visual pigment composition in the retina. Since the bleaching-resynthesis cycle takes place in the retina (Young, 1967; Bridges and Yoshikami, 1970c) a large increase in the supply of 3-dehydroretinol (Beatty, 1972; Jacquest and Beatty, 1972) could alter the ratio of the two vitamins (as prosthetic groups for visual pigment formation) in the ocular tissue thereby influencing visual pigment composition in the newly formed discs in the outer segments of the receptor cells (Young, 1967).

In conclusion, it seems evident, from the study, that visual pigment composition of rainbow trout is influenced by light, temperature and exogenous thyroxine. Both local and central mechanisms may co-exist in determining visual pigment composition in the retina. The properties of the hypothesized 3-dehydrogenase enzyme hold the key for understanding the apparent effect of light and exogenous thyroxine in different fishes. Homogenates prepared from pigment epithelium, retina and other ocular tissue from different fishes could lead to more information on the properties of this enzyme. Local administration of proper enzyme inhibitors to the ocular tissue of different fishes might also be able to assist in the elucidation of the physiological or biochemical processes involved. Until more research is done, evidence existing is far from sufficient to satisfactorily formulate any models for the mechanism of visual pigment conversion.

# BIBLIOGRAPHY

Ali, M. A. and H. J. Wagner. 1975. Visual Pigments: Phylogeny and Ecology. In: *Vision in Fishes*. Ed. by M. A. Ali. New York: Plenum Press (pp. 481-516).

Allen, D. M. 1970. Photic control of the proportions of the two visual pigments in a fish. Ph.D. Dissertation. University of Oregon, Eugene, Oregon.

Allen, D. M. 1971. Photic control of the proportions of the two visual pigments in a fish. Vision Res. 11:1077-1112.

Allen, D. M. and W. N. McFarland. 1973. The effect of temperature on rhodopsin-porphyropsin ratios in a fish. Vision Res. 13:1303-1309.

Allen, D. M., W. N. McFarland, F. W. Munz and M. A. Poston. 1973. Changes in the visual pigment of trout. Can. J. Zool. 51(9):901-914.

Beatty, D. D. 1966. A study of the succession of visual pigments in Pacific salmon (*Oncorhynchus*). Can. J. Zool. 44:429-455.

Beatty, D. D. 1969. Visual pigment changes in juvenile kokanee salmon in response to thyroid hormones. Vision Res. 9:855-864.

Beatty, D. D. 1972. Visual pigment changes in salmonid fishes in response to exogenous L-thyroxine, bovine TSH and 3-dehydroretinol. Vision Res. 12:1947-1960.

Beatty, D. D. 1975. Rhodopsin-porphyropsin changes in paired-pigment fishes. In: *Vision in Fishes*. Ed. by M. A. Ali. New York: Plenum Press (pp. 635-644).

Bownds, D., A. Brodie, W. E. Robinson, D. Palmar, J. Miller and A. Shedlovsky. 1974. Physiology and Enzymology of frog photoreceptor membranes. Exp. Eye Res. 18:253-269.

Braekkan, O. R., O. Ingebrigtsen and H. Myklestad. 1969. Uptake and storage of Vitamin A in rainbow trout, *Salmo gairdneri*. Internat. J. Vit. Res. 39(2):123-130.

Braverman, L. E., A. G. Vagenakis, A. E. Foster and S. H. Ingbar. 1971. Evaluation of a simplified technique for the specific measurement of a serum thyroxine concentration. J. Clin. Endocrinol. Metab. 32: 497-502.

Bridges, C. D. B. 1956. The visual pigments of the rainbow trout (*Salmo irideus*). J. Physiol. 134:620-629.

Bridges, C. D. B. 1964. Variation of visual pigment amongst individuals of an American minnow, *Notemigonus crysoleucas bosccii*. Vision Res. 4:233-239.

Bridges, C. D. B. 1965. Absorption properties, interconversions and environmental adaptation of pigments from fish photoreceptors. Cold Spr. Harb. Symp. Quant. Biol. 30:317-344.

Bridges, C. D. B. 1972. The rhodopsin-porphyropsin visual system. In: *Handbook of Sensory Physiology VII/I. Photochemistry of Vision*. Ed. by H. J. A. Dartnall. New York: Springer-Verlag (pp. 417-480).

Bridges, C. D. B. and C. E. Delisle. 1974. Postglacial evolution of the visual pigment of the smelt, *Osmerus eperlanus mordax*. Vision Res. 14:345-356.

Bridges, C. D. B. and S. Yoshikami. 1970a. Distribution and evolution of the visual pigments in salmonid fishes. Vision Res. 10:609-626.

Bridges, C. D. B. and S. Yoshikami. 1970b. The rhodopsin-porphyropsin system in fresh water fishes. 1. Effects of age and photic environment. Vision Res. 10:1315-1332.

Bridges, C. D. B. and S. Yoshikami. 1970c. The rhodopsin-porphyropsin system in fresh water fishes. 2. Turnover and interconversion of visual pigment prosthetic groups in light and darkness - role of the pigment epithelium. Vision Res. 10:1333-1345.

Crescitelli, F. 1956. The nature of the lamprey visual pigment. J. gen. Physiol. 39:423-435.

Cristy, M. 1974. Effects of prolactin and thyroxine on the visual pigments of trout, *Salmo gairdneri*. Gen. Comp. Endocrinol. 23:58-62.

Dartnall, H. J. A. 1957. *The Visual Pigments*. G.B.: Pitman Press.

Dartnall, H. J. A. 1962. The photobiology of visual processes. In: *The Eye, 2*. Ed. by H. Davson. New York: Academic Press (pp. 323-533).

Dartnall, H. J. A. 1968. The photosensitivities of visual pigments in the presence of hydroxylamine. Vision Res. J. 339-358.

Dartnall, H. J. A., M. R. Lander and F. W. Munz. 1961. Periodic changes in the visual pigments of a fish. In: *Progress in Photobiology*. Ed. by B. Chr. Christensen and B. Buckmann. Amsterdam: Elsevier (pp. 203-213).

Drujan, B. D., R. Castillion and E. Guerrero. 1968. Application of fluorometry in the determination of Vitamin A. Anal. Biochem. 23: 44-52.

Eales, J. G. 1974. Creation of chronic physiological elevations of plasma thyroxine in brook trout, *Salvelinus fontinalis* (Mitchill) and other teleosts. Gen. Comp. Endocrinol. 22:209-217.

Etkin, W. and A. G. Gona. 1974. Evolution of thyroid function in poikilotherm vertebrates. In: *Handbook of Physiology, Sect. 7 Endocrinology. Vol. III Thyroid.* Ed. by M. A. Greer and D. H. Solomon. Washington, D.C.: Amer. Physiol. Soc. (pp. 5-20).

Frank, R. N. and R. E. Bensinger. 1974. Rhodopsin and light-sensitive kinase activity of retinal outer segments. Exp. Eye Res. 18:271-280.

Freed, M. 1966. *Methods of Vitamin Assay.* New York: J. Wiley and Sons.

Garry, P. J., J. D. Pollack and G. M. Owen. 1970. Plasma Vitamin A assay by fluorometry and use of a silicic acid column technique. Clin. Chem. 16:766-772.

Hall, M. O. and D. Bok. 1974. Incorporation of [$^3$H] vitamin A into rhodopsin in light and dark adapted frogs. Exp. Eye Res. 18:105-117.

Hansen, L. G. and W. J. Warwick. 1968. A fluorometric micromethod for serum vitamin A. Amer. J. Clin. Pathol. 50:525-529.

Higgs, D. A. and J. G. Eales. 1973. Measurement of circulatory thyroxine in several fresh water teleosts by competitive binding analysis. Can. J. Zool. 51:49-53.

Hochachka, P. W. and G. N. Somero. 1973. *Strategies of Biochemical Adaptation.* Toronto: W. B. Saunders.

Hubbard, R., P. K. Brown and D. Bownd. 1971. Methodology of Vitamin A and visual pigment. In: *Methods of Enzymology. Vol. 18 Vitamins and Coenzymes. Part C.* Ed. by D. B. McCormick and L. D. Wright. New York: Academic Press (pp. 615-653).

Jacquest, W. L. 1969. Changes in the visual pigment composition of the rainbow trout, *Salmo gairdneri.* M.Sc. thesis. University of Alberta, Edmonton.

Jacquest, W. L. and D. D. Beatty. 1972. Visual pigment changes in the rainbow trout. Can. J. Zool. 50(8):1117-1126.

Kahan, J. 1966. A method for the fluorometric determination of vitamin A. Scandinav. J. Clin. Invest. 18:679-690.

Lythgoe, J. N. 1972. List of vertebrate visual pigments. In: *Handbook of Sensory Physiology VII/I. Photochemistry of Vision.* Ed. by H. J. A. Dartnall. New York: Springer-Verlag (pp. 604-623).

Moore, T. 1957. *Vitamin A.* New York: Elsevier.

Munz, F. W. and D. D. Beatty. 1965. A critical analysis of the visual pigments of salmon and trout. Vision Res. 5:1-17.

Munz, F. W. and W. N. McFarland. 1965. A suggested hereditary mechanism for visual pigments of the chars (*Salvelinus* spp.). Nature (London) 206:955-956.

Munz, F. W. and R. T. Swanson. 1965. Thyroxine-induced analysis of the visual pigments of salmon and trout. Amer. Zool. 5(4):683.

Murphy, B. E. P. and C. J. Pattee. 1964. Determination of thyroxine utilizing the property of protein binding. J. Clin. Endocrinol. Metab. 24:187-196.

Naito, K. and F. H. Wilt. 1962. The conversion of vitamin A to retinene$_2$ in a fresh water fish. J. Biol. Chem. 237:3060-3064.

Ohtsu, K., K. Naito and F. H. Wilt. 1964. Metabolic basis of visual pigment conversion in metamorphosing *Rana catesbeiana.* Develop. Biol. 10:216-232.

Olson, J. A. 1969. Metabolism and Function of Vitamin A. Federation Proceedings: Nutrition Society Symposium 28(5):1670-1677.

Plack, P. A. and P. M. J. Woodhead. 1966. Vitamin A compounds and lipids in the blood of the Cod, *Gadus morhua* from the arctic in relation to gonadal maturation. J. Mar. Biol. Ass. U.K. 46:547-559.

Pollack, J. D., G. W. Owen, P. J. Garry and D. Clark. 1973. Plasma retinol assay by elution from silicic acid with cymene (P-Isopropyl Toluene). Clin. Chem. 19(9):977-983.

Saunders, J. T. and S. M. Manton. 1969. *A Manual of Practical Vertebrate Morphology* (4th Ed.). Oxford: Clarendon Press (pp. 22-23).

Schwanzara, S. A. 1967. The visual pigments of fresh water fishes. Vision Res. 7:121-148.

Selvaraj, J. and T. P. Susheela. 1970. Estimation of serum vitamin A by a microfluorometric procedure. Clinica Chemica Acta 27:165-170.

Shantz, E. M. 1948. Isolation of pure vitamin $A_2$. Science (N.Y.) 108: 417-419.

Sokal, R. R. and F. J. Rohlf. 1969. *Biometry: The Principle and Practice of Statistics in Biological Research.* San Francisco: W. H. Freeman and Co.

Thompson, J. N., P. Erdody, R. Brien and T. K. Murray. 1971. Fluorometric determination of vitamin A in human blood and liver. Biochem. Med. 5(1):67-89.

Wald, G. 1939. On the distribution of vitamin $A_1$ and $A_2$. J. Gen. Physiol. 22:391-415.

Wald, G. 1957. The metamorphosis of visual systems in the sea lamprey. J. Gen. Physiol. 25:331-336.

Wilt, F. H. 1959. The organ specific action of thyroxine in visual pigment differentiation. J. Embryol. exp. Morphol. 7:556-563.

Yamada, E. 1961. The fine structure of the pigment epithelium in the turtle eye. In: *The Structure of the Eye*. Ed. by G. K. Smelser. New York: Academic Press (pp. 73-84).

Young, R. W. 1967. The renewal of photoreceptor cell outer segments. J. Cell Biol. 33:61-72.

# APPENDIX 1

## EFFECT OF EYE REMOVAL OPERATION ON VISUAL PIGMENT COMPOSITION

In order to avoid the problem of individual variation of visual pigment composition in rainbow trout, Jacquest and Beatty (1972) removed one eye of the fish at the beginning of their experiments and later removed the other. Assuming the percent $VP_2$ in the two eyes is the same at any time (Jacquest, 1969; Bridges, 1964), comparing the percent $VP_2$ from the left eye removed at the beginning of the experiment to that from the right eye at the end, one can get a more accurate estimation of any change in the proportion of porphyropsin during the experiment. However, eye removal could be a major trauma to the fish, accompanied by a considerable amount of blood loss. In order to show that the eye removal operation by itself does not impose any influence on visual pigment composition, some results from a number of pilot experiments are used.

At the beginning of each experiment, left eyes were removed from a number of fish randomly selected from a stock group of fish. These one-eyed fish were then subjected to the described (Appendix 1, Table 1) experimental conditions together with the two-eyed fish from the same stock group. Assuming the visual pigment composition of the one-eyed fish is not significantly different from the rest of the population at the beginning of the experiment, comparison of the visual pigment composition of right eyes of these fish to those of the two-eyed fish at the end of the experiment would indicate whether the eye removal operation had significantly influenced the visual pigment composition. Student's T test was used. The validity of using parametric tests on values of percent $VP_2$ has been confirmed (Appendix 3). Results accumulated from 95 fish show that the eye removal operation is not likely to significantly influence visual pigment composition in the fish.

Table 1

| Total no. of fish involved* | Experimental conditions | Interpretation of T test results |
|---|---|---|
| 8 | 12L/12D, 15° C, 15 days | N.S. |
| 8 | 12L/12D, 15° C, 30 days | N.S. |
| 8 | 12L/12D, 7° C, 15 days | N.S. |
| 11 | 12L/12D, 15° C, 15 days | N.S. |
| 9 | 24D, 15° C, 15 days | S. |
| 18 | 24D, 15° C, 30 days | N.S. |
| 9 | 24D, 7° C, 15 days | N.S. |
| 10 | 24D, 7° C, 30 days | N.S. |
| 8 | Room light, 7° C, 15 days | N.S. |
| 6 | Room light, 7° C, 30 days | N.S. |

N.S. - Not significantly different.

S. - Significantly different.

*The number of one-eyed fish plus the number of two-eyed fish.

# APPENDIX 2

## DETERMINATION OF VISUAL PIGMENT COMPOSITION IN RETINAL EXTRACT*

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*The experimental procedures were carried out under dim red light (Kodak Safelight with Wratten Series 2 filter ).

The frozen retinal extracts and the corresponding blank solution were thawed, agitated with a Vortex mixer and centrifuged at 10,000 XG for 10 minutes (at 20° C). The supernatants were transferred to optical cuvettes containing 0.05 ml of 0.2 M neutralized hydroxylamine. The measurement of the initial absorbance spectrum was carried out in a Cary model 14 recording spectrophotometer, recording from 700 nm to 350 nm at a scanning speed of 10 nm per second. An example of this initial spectrum is shown in Figure 1 of Appendix 2 (curve 1). The sample cuvette was then transferred to a specially constructed bleaching apparatus where the retinal extract was exposed to 10 minutes of orange light ($\lambda_{max}$ = 610 nm; quartz-iodine lamp, with an interference filter[1] passing light with a half band width of 4% of $\lambda_{max}$). The cuvette was then returned to the spectrophotometer where the density spectrum was recorded as before (Fig. 1, Appendix 2, curve 2). The pH of each retinal extract was measured at the end of the experiment.

Munz and Beatty (1965) prepared a series of template curves relating the wavelengths of the 90, 80, 70, . . . 10% points of the maximum absorbance change of a difference spectrum to various proportions of $VP503_1$ and $VP527_2$, represented as percent $VP527_2$ (100%, 90%, 80%, . . . 0%), in a mixture. Munz and Beatty (1965) found that the estimated percent $VP_2$ based on the wavelength of the 50% point of the total difference spectrum was as reliable as that based on the mean from the 90, 80, 70, . . . 10% points.

Therefore, a table (Table 1, Appendix 2) was prepared giving the percent $VP_2$ (based on absorbance) for the various wavelengths of the 50% point of the maximum absorbance change (from the total difference

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[1] Baird-Atomic Inc., Cambridge, U.S.A.

Figure 1. The initial, final and difference spectrum of a retinal extract prepared from one rainbow trout retina . Curve 1 indicates the initial spectrum of the retinal extract in digitonin solution (pH = 8.5). Curve 2 (final spectrum) was obtained after the retinal extract was exposed to orange light ($\lambda_{max}$ = 610) for 10 minutes. Curve 1-curve 2 gave the difference spectrum, curve 3.

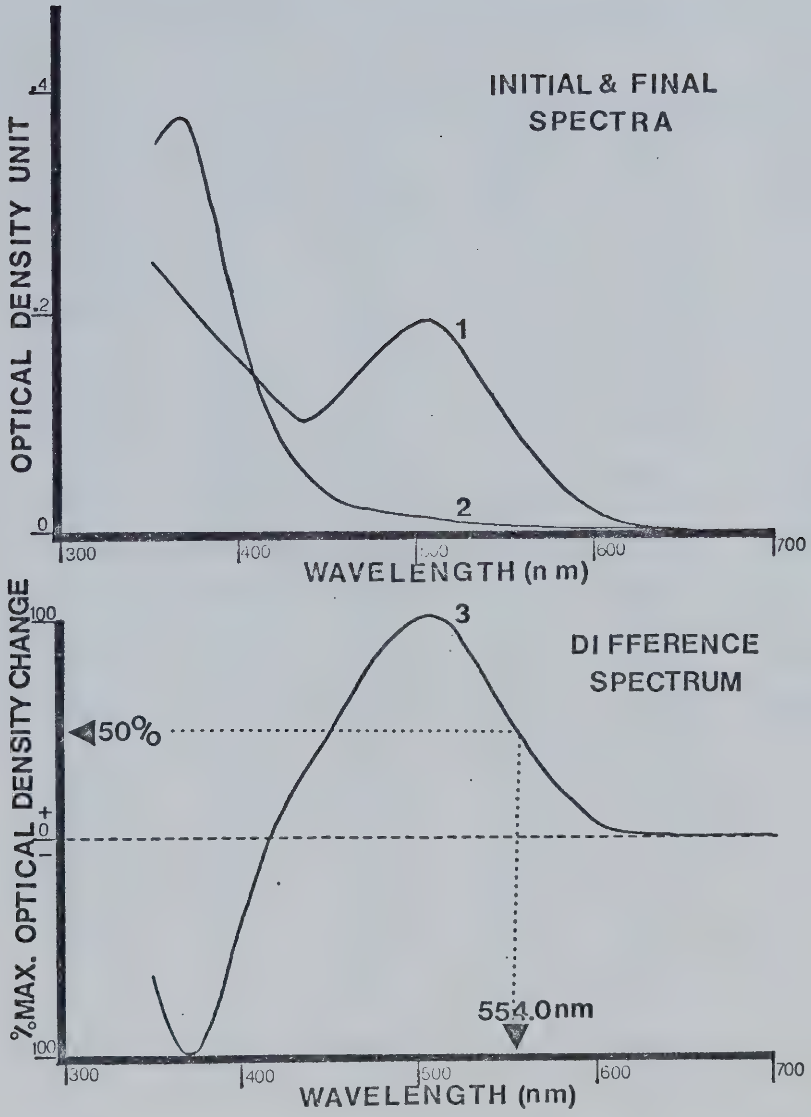
INITIAL & FINAL SPECTRA
OPTICAL DENSITY UNIT
.4
.2
0
1
2
300
400
500
600
700
WAVELENGTH (n m)
%MAX. OPTICAL DENSITY CHANGE
100
0
+
-
100
3
DIFFERENCE SPECTRUM
50%
554.0nm
300
400
500
600
700
WAVELENGTH (nm)

Table 1

Table giving percent VP$_2$ (column B, based on absorbance) for the various wavelengths (column A) at the 50% points of the maximum absorbance change (from the total difference spectrum)

| A | B | A | B | A | B | A | B |
|---|---|---|---|---|---|---|---|
| 550.0 | 1.0% | 560.0 | 32.3% | 570.0 | 55.9% | 580.0 | 82.2% |
| 550.5 | 2.1% | 560.5 | 33.3% | 570.5 | 57.1% | 580.5 | 83.8% |
| 551.0 | 4.1% | 561.0 | 35.0% | 571.0 | 58.8% | 581.0 | 85.6% |
| 551.5 | 6.9% | 561.5 | 36.0% | 571.5 | 59.2% | 581.5 | 87.4% |
| 552.0 | 7.2% | 562.0 | 37.4% | 572.0 | 60.5% | 582.0 | 89.4% |
| 552.5 | 8.9% | 562.5 | 38.1% | 572.5 | 61.9% | 582.5 | 91.1% |
| 553.0 | 11.4% | 563.0 | 40.0% | 573.0 | 63.0% | 583.0 | 92.7% |
| 553.5 | 13.0% | 563.5 | 40.9% | 573.5 | 64.0% | 583.5 | 95.0% |
| 554.0 | 14.0% | 564.0 | 41.8% | 574.0 | 65.8% | 584.0 | 96.0% |
| 554.5 | 16.9% | 564.5 | 42.5% | 574.5 | 66.4% | 584.5 | 98.0% |
| 555.0 | 18.0% | 565.0 | 43.6% | 575.0 | 67.9% | 585.0 | 100.0% |
| 555.5 | 19.7% | 565.5 | 45.1% | 575.5 | 69.1% | | |
| 556.0 | 20.5% | 566.0 | 46.1% | 576.0 | 70.8% | | |
| 556.5 | 22.1% | 566.5 | 47.6% | 576.5 | 71.9% | | |
| 557.0 | 23.9% | 567.0 | 49.0% | 577.0 | 73.3% | | |
| 557.5 | 25.4% | 567.5 | 49.7% | 577.5 | 74.5% | | |
| 558.0 | 26.1% | 568.0 | 51.0% | 578.0 | 76.1% | | |
| 558.5 | 27.9% | 568.5 | 52.0% | 578.5 | 77.6% | | |
| 559.0 | 29.1% | 569.0 | 53.2% | 579.0 | 79.0% | | |
| 559.5 | 30.4% | 569.5 | 54.2% | 579.5 | 80.0 | | |

spectrum).

The difference spectrum (curve 1 - curve 2) is represented by curve 3 (Appendix 2, Fig. 1). The 50% point of the total difference spectrum was located at 554 nm. From Table 1 (Appendix 2), this retinal extract was estimated to have 14.0% $VP_2$.

# APPENDIX 3

## VALIDATION FOR THE USE OF PARAMETRIC TESTS IN STATISTICAL ANALYSIS OF DATA OBTAINED IN THE STUDY

Allen (1970) showed that in the red-side shiner, *Richardsonius*, values of molar percent $VP_2$ obtained from the fish do not follow normal distribution when the means of the values get close to either end of the 0-100 percentage scale. His evidence was based on the result of the Kolmogorov-Smirnov test for goodness of fit to normal distribution on a group of 45 fish with a mean molar percent $VP_2$ of 9.8 ± 9.53 (Appendix C, Ph.D. Thesis, U. of Oregon, Allen, 1970). He found that by transforming the same data through an arcsine transformation, normal distribution of data can be achieved. Therefore he concluded that all data involving molar percent $VP_2$ should be transformed by arcsine transformation before statistical analysis using parametric tests is performed.

The molar percent $VP_2$ sampled in a number of groups in this study were tested for normal distribution using the same Kolmogorov-Smirnov tests. The results are shown in Table 1 (Appendix 3). No deviation from normality was found in each group irrespective of position of their mean percent $VP_2$ on the 0-100% scale. A total of 286 values were used in this analysis.

In addition, values of T4 concentration and percent 3-dehydroretinol were similarly tested for fitness to normal distribution. No significant deviation was found in results obtained.

Table 1

Result of Kolmogorov-Smirnov tests on values of molar percent $VP_2$ sampled at the beginning (left eyes) and end (right eyes) of each experiment

| Groups or experiments | Material analysed | Molar percent $VP_2$ Mean ± S.E. (n) | Interpretation of result* |
|---|---|---|---|
| Expt. 1 | Left eyes | 34.9 ± 1.4 (n=50) | + |
| Expt. 2 | Left eyes | 84.7 ± 0.7 (n=57) | + |
| Expt. 5 | Left eyes | 91.5 ± 0.5 (n=43) | + |
| Expt. 6 | Left eyes | 22.4 ± 2.4 (n=29) | + |
| Gp. 1-A | Right eyes | 45.5 ± 2.0 (n=11) | + |
| Gp. 1-B | Right eyes | 46.8 ± 3.8 (n=18) | + |
| Gp. 1-C | Right eyes | 85.2 ± 1.9 (n=12) | + |
| Gp. 1-D | Right eyes | 16.4 ± 2.4 (n=9) | + |
| Gp. 2-W | Right eyes | 70.9 ± 2.6 (n=11) | + |
| Gp. 2-X | Right eyes | 31.2 ± 3.5 (n=13) | + |
| Gp. 2-Y | Right eyes | 79.5 ± 1.5 (n=14) | + |
| Gp. 2-Z | Right eyes | 57.9 ± 2.1 (n=19) | + |

*Positive sign means normal distribution.

# APPENDIX 4*

## ISOLATION OF RETINOL FROM CAROTENOIDS USING SILICIC ACID COLUMN

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*See also Appendix 6 for fluorometric properties of retinol and 3-dehydroretinol.

Both Garry *et al.* (1970) and Pollack *et al.* (1973) suggested methods of isolation of vitamin A from human blood using a silicic acid microcolumn. Although the latter method claims specificity for retinol (see below) in blood, it required a large volume of plasma and therefore was not practical for this study. In order to establish the validity of the method of Garry *et al.* (1970) on plasma (pooled form) of rainbow trout, I attempted two individual tests.

1) Comparison of "Total vitamin A" versus "retinol" concentration in pooled plasma from rainbow trout

Since the method of Garry *et al.* (1970) measures total vitamin A (mostly retinol and retinol derivatives) in blood while the method of Pollack *et al.* (1973) measures specifically retinol, one should be able to estimate the amount of retinol derivatives in rainbow trout plasma simply by comparison of results obtained by the two methods from pooled plasma sample. Two determinations (total vitamin A and retinol) from the same pooled plasma sample agreed to within 3.8% indicating that any retinol derivatives, if present, do not show up in the assay (Garry *et al.*, 1970) employed in this study. Therefore, total vitamin A indicated by this test (Garry *et al.*, 1970) is recognized as retinol concentration.

2) Internal standard

The retinol concentration of pooled plasma from rainbow trout was determined (for extraction and fluorometric assay, see Materials and Methods; the method of Garry *et al.*, 1970, was used for isolation of retinol). A measured amount of retinol (Nutritional Biochemical) was dissolved into the plasma and the retinol content of the "enriched" plasma was redetermined. From the differences in the two determinations, one can calculate the percentage recovery of the added retinol, thereby

verifying the efficiency of the method with respect to extraction, isolation and detection of the retinol content in the plasma. A range of 75% to 90% recovery was obtained from two different pooled plasma samples of rainbow trout.

# APPENDIX 5*

## SEPARATION OF RETINOL AND 3-DEHYDRORETINOL IN PETROLEUM ETHER UTILIZING THE SILICIC ACID MICROCOLUMN

## (ADSORPTION CHROMATOGRAPHY)

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*See also Appendix 6 for fluorometric properties of retinol and 3-dehydroretinol.

The proof for separation of retinol and 3-dehydroretinol in petroleum ether by adsorption chromatography in a silicic acid column mainly comes from the percentage recovery of retinol and 3-dehydroretinol from the eluate. Garry *et al.* (1974) found that by dissolving retinol in petroleum ether and passing the solution through silicic acid column, all the dissolved retinol was retained in the column. However, 93 to 100% of the retained retinol could be recovered when proper amount of isopropanol was put through the column. This study confimed this phenomenon. An average percentage recovery of 102% was found.

When 3-dehydroretinol was dissolved in petroleum ether and the solution put through silicic acid column, all the 3-dehydroretinol in the solution was recovered from the petroleum ether eluate. This means that the silicic acid column has the property of selectively retaining retinol (see previous paragraph) but not 3-dehydroretinol in petroleum ether solution. A further attempt to confirm this finding was done by dissolving equivalent amounts of retinol and 3-dehydroretinol in petroleum ether and then determining the percentage recovery in each of the isopropanol and petroleum ether eluates. Assuming all fluorescence in petroleum ether came from 3-dehydroretinol, 137.2% recovery was calculated. Assuming all fluorescence in isopropanol eluate came from retinol, 110.52% recovery was found. The calculated percent 3-dehydroretinol was therefore 55%. This gives a percentage error of 10% which indicated a good estimation of the percent 3-dehydroretinol in the mixture.

The percentage recovery from each eluate, as one had noticed, was higher than 100% in each case. This was probably due to non-specific

interference substances from the silicic acid column. It was found that it could be lessened by washing the column three times with petroleum ether immediately before usage. This was done in all silicic acid columns used in this study.

# APPENDIX 6

## FLUOROMETRIC PROPERTIES OF RETINOL AND 3-DEHYDRORETINOL

A) Excitation and Emission Maxima of Retinol and 3-Dehydroretinol

B) Preparation of Standard Curves of Fluorescence at Different Concentration of Retinol and 3-Dehydroretinol in Petroleum Ether

## A. Excitation and Emission Maxima of Retinol and 3-Dehydroretinol

The excitation and emission maxima of retinol have been described by various workers (Kahan, 1966; Drujan *et al.*, 1968; Selvaraj and Susheela, 1970; Thompson *et al.*, 1971; Hansen and Warwick, 1968). However, none could be found for 3-dehydroretinol. In this study, repeated testing showed that in petroleum ether and isopropanol, the excitation maxima for retinol and 3-dehydroretinol ranged from 337 to 350 nm and 323 to 336 nm, respectively. They had common emission maxima at 490 nm.

Pure retinol was purchased as the crystalline alcohol,[1] the purity of which was confirmed by the Carr-Price test (Hubbard *et al.*, 1971) before it was used as a standard.

3-dehydroretinol was extracted from the retina of goldfish by the method described by Hubbard *et al.* (1971). Using the equation by Wald (1939), which was later modified by Wilt (1959), no detectable retinol or β-carotene was found in the extract examined by the Carr-Price Test.

The 3-dehydroretinol was extracted from the goldfish retina as follows:

> Goldfish were dark adapted for 2 hours before removal of eyes and dissection of the retina (under Kodak safelight, Wratten Series No. 2 filter). The dissected retinae were placed in phosphate buffer and exposed to orange light (610 nm) for half an hour at 20° C. They were left in the dark for another half an hour for the reduction of 3-dehydroretinal to 3-dehydroretinol. The buffer was removed by washing the retina three times with distilled water. The retinae were then ground in anhydrous sodium sulphate and the 3-dehydroretinol extracted with three 5 ml portions of diethyl ether. No saponification was performed afterwards. The solution was then evaporated to dryness under $N_2$ and redissolved in chloroform for Carr-Price test. It was found that

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[1] Nutritional Biochemical Corporation, Cleveland, Ohio 44128.

30 retinae provided an O.D. of 0.8, i.e., 1170.72 μg/100 ml of 3-dehydroretinol assuming $E_{1\,cm}^{1\%}$ is 4100 at 690 nm (Shantz, 1948). The extraction was carried out under a 40 W red bulb illumination.

### B. Preparation of Standard Curves at Different Concentrations of Retinol and 3-Dehydroretinol in Petroleum Ether

A small amount of retinol (0.5 mg Nutritional Biochemical) was completely dissolved in 10 ml diethyl ether. This was divided into two equal portions and placed inside a desiccator to evaporate to dryness under nitrogen. One portion was redissolved with 5 ml of chloroform and the other, with 5 ml of petroleum ether. The concentration of the chloroform portion was determined by Carr-Price test,[1] using the extinction coefficient of $E^{1\%}_{1\,cm}$ = 5070 at 620 nm for retinol (Moore, 1957). The petroleum ether portion was diluted appropriately and the fluorescence measured in the spectrofluorometer (Emission wavelength = 490; Excitation scan: 370-320 nm). Assuming the concentration of retinol in the chloroform solution is equal to that in the petroleum ether portion, a standard curve could then be established (see Figure 1, Appendix 6). [In order to confirm this assumption, some of the chloroform portion (used for Carr-Price test), on one occasion, was dried under $N_2$ and redissolved with an equivalent amount of petroleum ether. The fluorescent reading of this solution agreed with the petroleum ether portion to within 2.4%, showing that the concentration of the chloroform and petroleum ether portion is comparable.] In addition, retinol gives the same amount of fluorescence in petroleum ether as in isopropanol.

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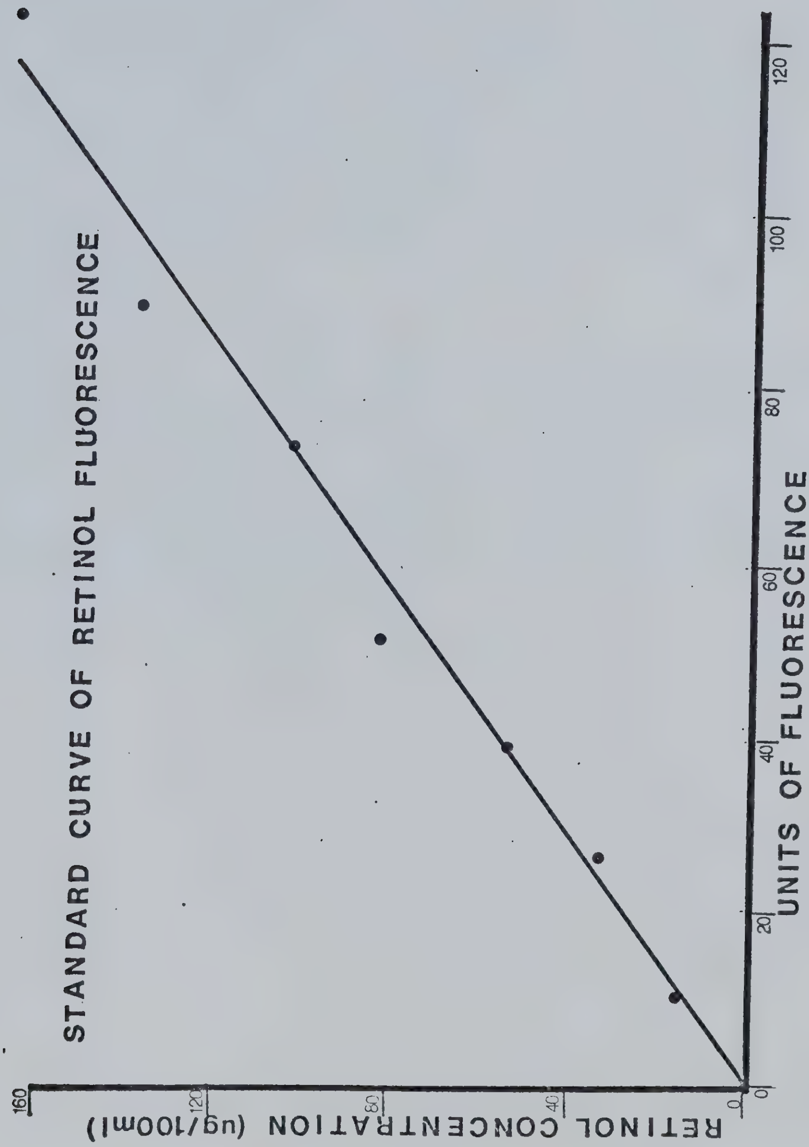
[1] 0.5 ml sample added to 2.5 ml saturated antimony trichloride containing two drops of acetic anhydride. The absorbance spectrum of the solution was recorded from the spectrophotometer within 30 seconds of preparation (Hubbard *et al.*, 1971).

Figure 1. Standard curve of retinol fluorescence. (For preparation procedures, see section B, Appendix 6.)

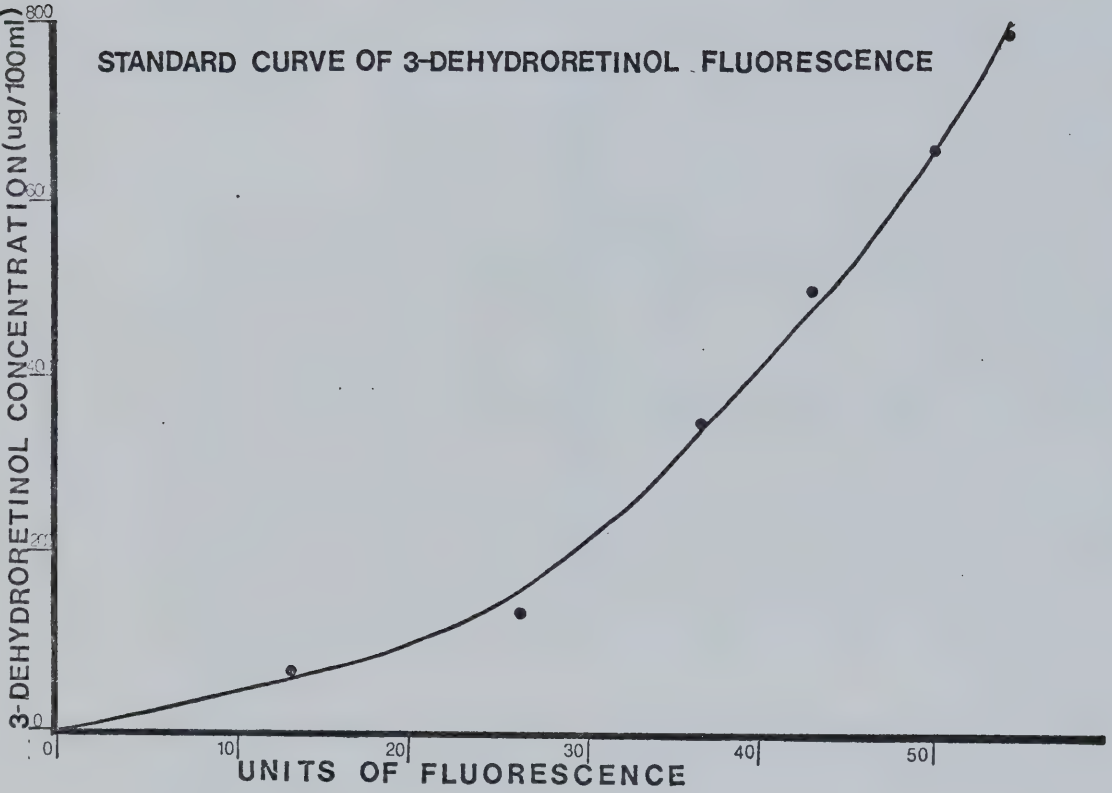


STANDARD CURVE OF RETINOL FLUORESCENCE

Pure 3-dehydroretinol was extracted from goldfish retina in the same way described in section A of this appendix. Sixty goldfish retinae were used and the diethyl ether extract was divided into two equal portions and evaporated to dryness under nitrogen. A standard curve (Fig. 2, Appendix 6) was similarly prepared by redissolving the two portions with chloroform and petroleum ether. The concentration of 3-dehydroretinol was determined from the chloroform portion by the Carr-Price test, assuming it has an extinction coefficient of $E^{1\%}_{1\,cm} = 4100$ at 690 nm (Shantz, 1948).

Comparison of the two standard curves shows that 3-dehydroretinol is a weak fluorescent compound in comparison to retinol (at 100 µg/100 ml, retinol fluoresces 3.5 times higher than 3-dehydroretinol). In addition, the fluorescence of 3-dehydroretinol does not increase linearly with the increase in 3-dehydroretinol concentration.

Figure 2. Standard curve of 3-dehydroretinol fluorescence. (For preparation procedures, see section B, Appendix 6)



STANDARD CURVE OF 3-DEHYDRORETINOL FLUORESCENCE

# APPENDIX 7

## NUMERICAL RESULTS FROM EXPERIMENTS 1 TO 7

Table 1

Experiment 1* (Group 1-A, 12L/12D, 16° C)

| | Weight (g) | | | $[T_4]^1$ | | Molar percent VP2 | | $\Delta D_{max}{}^2$ | |
|---|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | Initial | Final | Initial | Final | Initial | Final |
| A1** | 64 | / | / | 1.05 | / | / | / | / | / |
| A2** | 80 | / | / | 1.18 | / | 47.4 | / | .2383 | / |
| A3 | 68 | 51 | F | 0.00 | 1.32 | 29.8 | 44.7 | .3510 | .2543 |
| A4 | 69 | 55 | F | 1.28 | 1.61 | 49.3 | 55.1 | .2673 | .2430 |
| A5 | 61 | 53 | M | 1.61 | 0.56 | 25.9 | 47.4 | .3213 | .2263 |
| A6 | 56 | 47 | M | 0.98 | 1.39 | 35.7 | 48.4 | .3173 | .2028 |
| A7 | 57 | 48 | M | 0.62 | 1.91 | 35.7 | 49.3 | .3215 | .2300 |
| A8 | 67 | 54 | M | 0.00 | 1.58 | 31.5 | 45.4 | .2440 | .1600 |
| A9 | 55 | 52 | F | 1.10 | 2.35 | 25.9 | 29.8 | .3275 | .2659 |
| A10 | 50 | 39 | F | 0.17 | 1.45 | 22.9 | / | .3030 | / |
| A11 | 51 | 46 | M | 0.32 | 0.39 | 22.9 | 42.2 | .3735 | .2243 |
| A12 | 43 | 42 | M | / | 1.53 | / | 51.1 | / | .2055 |
| A13 | 49 | 41 | M | 1.27 | 1.13 | 51.1 | 45.4 | .3283 | .1495 |
| A14 | 48 | 48 | M | 2.35 | 1.36 | 31.5 | 42.2 | .3505 | .2225 |
| n | 14 | 12 | 12 | 13 | 12 | 12 | 11 | 12 | 11 |
| $\bar{X}$ | 58 | 48 | | 0.92 | 1.38 | 36.8 | 45.5 | .3161 | .2167 |

*Fish were preconditioned at 16° C in continuous total darkness for one month before the initial results were taken. They were then subjected to the indicated light and temperature regimes for 28 days before the final results were obtained.

**A1, A2 died.

[1] $[T_4]$ in μg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

## Table 2

Experiment 1* (Group 1-B, 24D, 16° C)

| | Weight (g) | | | $[T_4]$[1] | | Molar percent $VP_2$ | | $\Delta D_{max}$[2] | |
|---|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | Initial | Final | Initial | Final | Initial | Final |
| B1 | 69 | 70 | M | 1.86 | 0.59 | / | 29.8 | / | .2843 |
| B2** | 39 | / | / | 2.28 | / | 9.5 | / | .2438 | / |
| B3 | 61 | 60 | F | 0.85 | 1.50 | 35.7 | 18.1 | .1373 | .3915 |
| B4 | 51 | 51 | F | 1.30 | 1.16 | 25.9 | 9.5 | .2825 | .2758 |
| B5 | 37 | 36 | M | 0.86 | 1.77 | 11.7 | 5.5 | .2345 | .2345 |
| B6 | 54 | 53 | F | 1.12 | 1.44 | 29.8 | 16.8 | .3313 | .3555 |
| B7 | 48 | 47 | M | 0.85 | 0.35 | 35.7 | 11.7 | .1870 | .2290 |
| B8** | 64 | / | / | 1.24 | / | 40.3 | / | .1440 | / |
| B9 | 37 | 38 | M | 1.22 | 0.65 | 32.3 | 18.1 | .2780 | .2070 |
| B10 | 41 | 43 | M | 0.88 | 1.20 | 47.4 | 14.8 | .1428 | .3760 |
| B11** | 63 | / | / | 1.90 | / | 44.7 | / | .4110 | / |
| B12 | 38 | 34 | F | / | 0.58 | 34.4 | 22.9 | .2500 | .3500 |
| n | 12 | 9 | 9 | 11 | 9 | 11 | 9 | 11 | 9 |
| $\bar{X}$ | 50 | 48 | | 1.31 | 1.03 | 31.6 | 16.4 | .2402 | .3004 |

*Fish were preconditioned at 16° C in continuous total darkness for a month before the initial samples were taken. They were then subjected to the indicated light and temperature regimes for 28 days before the final samples were obtained.

**B2, B8, B11 died.

[1] $[T_4]$ in μg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 3

Experiment 1* (Group 1-C, 12L/12D, 6° C)

| | Weight (g) | | | $[T_4]$[1] | | Molar percent $VP_2$ | | $\Delta D_{max}$[2] | |
|---|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | Initial | Final | Initial | Final | Initial | Final |
| C1 | 41 | 48 | M | 1.23 | 1.46 | 40.3 | 91.9 | .2045 | .1390 |
| C2 | 62 | 65 | M | 1.24 | 0.16 | 34.4 | 84.4 | .3430 | .1620 |
| C3 | 61 | 66 | M | 1.17 | 1.84 | 35.7 | 88.9 | .3330 | .1958 |
| C4 | 47 | 49 | F | 0.66 | 0.34 | 35.7 | 76.6 | .2663 | .1093 |
| C5 | 57 | 60 | F | / | 1.29 | 42.2 | 88.9 | .1623 | .1108 |
| C6 | 67 | 74 | F | 1.23 | 0.77 | 34.4 | 86.2 | .2365 | .2115 |
| C7 | 69 | 73 | F | 0.25 | 1.51 | 18.1 | 91.9 | .3378 | .1833 |
| C8 | 39 | 46 | M | 1.12 | 0.35 | 25.9 | 90.4 | .2000 | .0988 |
| C9 | 44 | 51 | M | / | 0.88 | 27.7 | 87.5 | .1828 | .1520 |
| C10 | 48 | 54 | M | 1.93 | 0.26 | 32.3 | 69.7 | .2445 | .4245 |
| C11 | 52 | 60 | F | 0.59 | 0.00 | 22.9 | 82.4 | .3353 | .1583 |
| C12 | 68 | 73 | M | 0.29 | 1.40 | 40.3 | 83.6 | .3193 | .1345 |
| n | 12 | 12 | 12 | 10 | 12 | 12 | 12 | 12 | 12 |
| $\bar{X}$ | 55 | 60 | | 0.97 | 0.86 | 32.5 | 85.2 | .2637 | .1734 |

*Fish were preconditioned at 16° C in continuous total darkness for a month before the initial samples were taken. They were then subjected to the indicated light and temperature regimes for 28 days before the final samples were obtained.

[1] $[T_4]$ in μg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 4

Experiment 1* (Group 1-D, 24D, 6° C)

| | Weight (g) | | | $[T_4]$[1] | | Molar percent $VP_2$ | | $\Delta D_{max}$[2] | |
|---|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | Initial | Final | Initial | Final | Initial | Final |
| D1** | 40 | 41 | F | / | 0.15 | 43.2 | 49.3 | .1740 | .0938 |
| D2** | 34 | 33 | F | / | / | 39.2 | 35.7 | .2975 | .2093 |
| D3** | 51 | 59 | F | / | 0.37 | 39.2 | 69.7 | .1495 | .2178 |
| D4** | 37 | 39 | M | / | 0.42 | 43.2 | 35.7 | .2643 | .1808 |
| D5** | 59 | 70 | M | / | 0.86 | 22.9 | 57.2 | .1508 | .2235 |
| D6 | 54 | 58 | M | 1.54 | 1.63 | 44.7 | 68.7 | .1195 | .1983 |
| D7 | 55 | 67 | F | 2.55 | 0.97 | 35.7 | 22.9 | .3096 | .1930 |
| D8 | 61 | 61 | M | 0.73 | 0.19 | 29.8 | 35.7 | .2835 | .1603 |
| D9 | 37 | 36 | M | 0.87 | 1.58 | 22.9 | 47.4 | .2628 | .1783 |
| D10 | 53 | 54 | F | 4.10 | 1.48 | 43.2 | 44.7 | .4163 | .1843 |
| D11 | 49 | 51 | M | 0.80 | 1.24 | 29.8 | 47.4 | .2223 | .2230 |
| D12 | 35 | 35 | F | 0.32 | 1.23 | 32.3 | 74.1 | .3153 | .1983 |
| D13 | 77 | 81 | F | 1.64 | 0.89 | 32.3 | 27.7 | .3095 | .2000 |
| D14 | 51 | 56 | M | 0.74 | 2.00 | 58.1 | 72.8 | .3855 | .1935 |
| D15 | 57 | 57 | M | / | 0.16 | 44.7 | 35.7 | .2055 | .2708 |
| D16 | 48 | 47 | F | 1.77 | 0.35 | 45.4 | 51.1 | .2043 | .2240 |
| D17 | 50 | 59 | M | 0.12 | 0.98 | 61.6 | 39.2 | .3568 | .3593 |
| D18 | 41 | 44 | F | / | 1.72 | 39.2 | 27.4 | .3090 | .1050 |
| n | 18 | 18 | 18 | 11 | 17 | 18 | 18 | 18 | 18 |
| $\bar{X}$ | 49 | 53 | | 1.38 | 0.96 | 39.3 | 46.8 | .2653 | .2007 |

*Fish were preconditioned at 16° C in continuous total darkness for a month before the initial samples were taken. They were then subjected to the indicated light and temperature regimes for 28 days before the final samples were obtained.

**D1-D5 were not bled at the beginning of experiment.

[1] $[T_4]$ in μg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 5

Experiment 2* (Group 2-W, 24D, 6° C)

| | Weight (g) | | | $[T_4]^1$ | | Molar percent $VP_2$ | | $\Delta D_{max}{}^2$ | |
|---|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | Initial | Final | Initial | Final | Initial | Final |
| W1 | 60 | 49 | F | 1.65 | 0.56 | 87.5 | 70.6 | .1770 | .0318 |
| W2 | 85 | 80 | F | 2.58 | 0.37 | 90.5 | 77.6 | .1380 | .1983 |
| W3** | 70 | / | / | 0.15 | / | 81.2 | / | .1758 | / |
| W4** | 52 | / | / | 0.00 | / | 84.4 | / | .2370 | / |
| W5 | 52 | 57 | F | 3.71 | 0.60 | 78.8 | 75.2 | .2222 | .1885 |
| W6 | 92 | 81 | F | 1.22 | 1.02 | 87.5 | 74.1 | .1593 | .2175 |
| W7 | 40 | 44 | M | 0.71 | 1.07 | 90.4 | 78.8 | .1935 | .1430 |
| W8 | 49 | 47 | F | 1.82 | 0.60 | 88.9 | 76.6 | .1770 | .1683 |
| W9** | 51 | / | / | 0.85 | / | 91.9 | / | .1945 | / |
| W10 | 76 | 70 | F | 2.90 | 1.48 | 83.6 | 63.2 | .2425 | .2159 |
| W11 | 5] | 56 | M | 1.22 | 0.94 | 88.9 | 72.3 | .1703 | .1455 |
| W12 | 58 | 55 | F | 1.53 | 1.59 | 88.9 | 78.8 | .2033 | .1670 |
| W13 | 72 | 72 | M | 1.60 | 0.13 | 82.4 | 53.7 | .2065 | .2260 |
| W14 | 64 | 58 | F | 0.50 | 1.47 | 86.2 | 58.8 | .2260 | .1280 |
| n | 14 | 11 | 11 | 14 | 11 | 14 | 11 | 14 | 11 |
| $\bar{X}$ | 62 | 61 | | 1.46 | 0.89 | 86.5 | 70.9 | .1945 | .1798 |

*Initial samples were obtained upon arrival of fish from the rearing station. They were then subjected to the indicated light and temperature regimes for 28 days before the final samples were obtained.

**W3, W4, W9 died.

[1] $[T_4]$ in µg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 6

Experiment 2* (Group 2-X, 24D, 16° C)

| | Weight (g) | | | $[T_4]$[1] | | Molar percent $VP_2$ | | $\Delta D_{max}$[2] | |
|---|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | Initial | Final | Initial | Final | Initial | Final |
| X1 | 96 | 66 | M | 1.80 | 0.97 | 82.4 | 18.1 | .2285 | .2210 |
| X2 | 75 | 88 | M | 2.15 | / | 76.6 | 42.2 | .1750 | .1689 |
| X3 | 66 | 56 | F | 0.47 | 0.39 | 78.8 | 34.4 | .1798 | .1938 |
| X4 | 62 | 52 | F | 1.92 | 1.25 | 82.4 | 25.9 | .2063 | .3248 |
| X5 | 53 | 51 | F | / | 1.42 | 93.3 | 11.7 | .1958 | .1900 |
| X6 | 64 | 67 | M | 5.32 | 1.50 | 83.6 | 14.8 | .1783 | .3640 |
| X7 | 77 | 74 | F | 1.20 | 1.02 | 88.9 | 29.8 | .2330 | .2125 |
| X8 | 45 | 50 | F | 4.52 | 0.98 | 84.4 | 29.8 | .1350 | .2958 |
| X9 | 55 | 56 | M | 3.51 | 0.79 | 87.5 | 34.4 | .1883 | .2418 |
| X10 | 82 | 71 | F | 4.25 | 0.38 | 91.9 | 92.2 | .2083 | .3523 |
| X11 | 50 | 41 | M | 1.11 | 0.61 | 72.3 | 51.1 | .2005 | .1380 |
| X12 | 63 | 49 | F | 1.14 | 1.33 | 83.6 | 49.3 | .2113 | .2823 |
| X13 | 54 | 54 | F | 3.33 | 1.11 | 86.2 | 21.6 | .1718 | .3328 |
| X14** | 65 | / | / | 0.00 | / | 87.5 | / | .1835 | / |
| n | 14 | 13 | 13 | 13 | 12 | 14 | 13 | 14 | 13 |
| $\bar{X}$ | 63 | 60 | | 2.36 | 0.98 | 84.2 | 31.2 | .1925 | .2553 |

*Initial samples were obtained upon arrival of fish from the rearing station. They were then subjected to the indicated light and temperature regimes for 28 days before the final samples were obtained.

**X14 died.

[1] $[T_4]$ in μg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 7

Experiment 2* (Group 2-Y, 12L/12D, 6° C)

| Code | Weight (g) |  | Sex | $[T_4]$[1] |  | Molar percent $VP_2$ |  | $\Delta D_{max}$[2] |  |
|---|---|---|---|---|---|---|---|---|---|
|  | Initial | Final |  | Initial | Final | Initial | Final | Initial | Final |
| Y1 | 57 | 55 | M | 0.05 | 0.19 | 78.8 | 81.2 | .6620 | .1200 |
| Y2 | 62 | 59 | M | 0.71 | 1.37 | 83.6 | 78.8 | .2355 | .1313 |
| Y3 | 61 | 64 | M | 0.35 | 0.98 | 84.4 | 72.3 | .0781 | .1455 |
| Y4 | 60 | 62 | F | 0.73 | 0.29 | 86.2 | 84.4 | .0559 | .1383 |
| Y5 | 82 | 86 | M | 0.78 | 0.54 | 90.4 | 88.9 | .1720 | .1330 |
| Y6 | 78 | 75 | F | 3.63 | 1.25 | 93.3 | 86.2 | .2125 | .0546 |
| Y7 | 46 | 47 | M | 1.52 | 1.03 | 81.2 | 78.8 | .1500 | .0793 |
| Y8 | 55 | 51 | F | / | 0.97 | 82.4 | 78.8 | .1928 | .1080 |
| Y9 | 50 | 45 | F | 1.58 | 0.35 | 68.7 | 75.2 | .1540 | .1515 |
| Y10 | 74 | 68 | M | 1.57 | 1.38 | 78.8 | 69.7 | .1580 | .1420 |
| Y11 | 66 | 62 | F | 1.86 | 1.09 | 84.4 | 78.8 | .1938 | .1883 |
| Y12 | 51 | 47 | M | 1.05 | 1.44 | 76.6 | 74.1 | .1508 | .1150 |
| Y13 | 43 | 39 | F | 2.02 | 0.98 | 83.6 | 84.4 | .1540 | .1023 |
| Y14 | 73 | 74 | F | 0.35 | 1.25 | 77.6 | 81.2 | .2910 | .1515 |
| n | 14 | 14 | 14 | 13 | 14 | 14 | 14 | 14 | 14 |
| $\bar{X}$ | 61 | 60 |  | 1.25 | 0.94 | 82.1 | 79.5 | .1615 | .1258 |

*Initial samples were obtained upon arrival of fish from the rearing station. They were then subjected to the indicated light and temperature regimes for 28 days before the final samples were obtained.

[1] $[T_4]$ in µg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 8

Experiment 2* (Group 2-Z, 12L/12D, 16° C)

| | Weight (g) | | | $[T_4]$[1] | | Molar percent $VP_2$ | | $\Delta D_{max}$[2] | |
|---|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | Initial | Final | Initial | Final | Initial | Final |
| Z1 | 64 | 63 | M | 2.52 | 1.47 | 83.6 | 69.7 | .2055 | .1320 |
| Z2 | 61 | 57 | F | 0.93 | 1.34 | 91.9 | 65.9 | .1555 | .1680 |
| Z3 | 62 | 85 | F | 2.21 | 1.14 | 77.6 | 50.0 | .2275 | .2013 |
| Z4 | 79 | 74 | M | / | 0.28 | 88.9 | 67.5 | .1708 | .1973 |
| Z5 | 55 | 50 | F | 3.93 | 0.28 | 90.4 | 60.6 | .1925 | .1468 |
| Z6 | 77 | 73 | M | 1.74 | 0.23 | 93.3 | 70.6 | .2465 | .1765 |
| Z7 | 99 | 84 | F | 3.02 | 1.56 | 88.9 | 64.3 | .2270 | .1720 |
| Z8 | 39 | 36 | F | 1.24 | 0.10 | 84.4 | 63.2 | .1893 | .1438 |
| Z9 | 54 | 40 | M | 2.30 | 1.14 | 86.2 | 50.0 | .2135 | .1310 |
| Z10 | 38 | 41 | F | 1.65 | 0.42 | 81.2 | 45.4 | .1740 | .1803 |
| Z11 | 81 | 70 | M | 5.72 | 1.73 | 78.8 | 60.6 | .1775 | .1973 |
| Z12 | 91 | 79 | F | 1.77 | 1.45 | 88.9 | 61.6 | .2375 | .2345 |
| Z13 | 89 | 88 | M | 0.50 | 0.31 | 84.4 | 44.7 | .2310 | .2113 |
| Z14 | 60 | 48 | F | 6.00 | 1.20 | 83.6 | 52.7 | .1995 | .1665 |
| Z15** | 64 | 59 | F | / | 1.32 | 82.4 | 42.2 | .1870 | .2040 |
| Z16** | 69 | 63 | M | / | 0.99 | 83.6 | 58.5 | .1575 | .1628 |
| Z17** | 49 | 44 | M | / | 1.78 | 82.4 | 64.3 | .1810 | .1333 |
| Z18** | 42 | 34 | F | / | 3.97 | 87.5 | 43.2 | .1670 | .1568 |
| Z19** | 62 | 53 | F | / | 1.46 | 88.9 | 63.2 | .1653 | .1690 |
| n | 19 | 19 | 19 | 13 | 19 | 19 | 19 | 19 | 19 |
| $\bar{X}$ | 65 | 60 | | 2.12 | 1.17 | 85.6 | 57.9 | .1950 | .1703 |

*Initial samples were obtained upon arrival of fish from the rearing station. They were then subjected to the indicated light and temperature regimes for 28 days before the final samples were obtained.

**Z15-Z19 were not bled at the beginning of the experiment.

[1] $[T_4]$ in μg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

## Table 9

### Experiment 3* (Group 3-L, 12L/12D)

| | Weight (g) | | | Time of Sampling (Days) | Molar percent $VP_2$ | | $\Delta D_{max}$*** | |
|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | | Initial | Final | Initial | Final |
| L1 | 101 | 85 | M | 24 | 91.9 | 77.6 | .2455 | .1778 |
| L2 | 91 | 67 | F | 30 | 78.8 | 64.3 | .1616 | .1550 |
| L3** | 82 | / | / | / | 73.3 | / | .1960 | / |
| L4 | 86 | 71 | M | 15 | 78.8 | 67.5 | .1958 | .2173 |
| L5 | 98 | 80 | F | 6 | 88.9 | 86.2 | .2120 | .1810 |
| L6 | 93 | 81 | M | 9 | 86.2 | 75.2 | .2055 | .2195 |
| L7** | 74 | // | / | / | 71.9 | / | .6920 | / |
| L8 | 85 | 75 | M | 15 | 83.6 | 66.3 | .2293 | .1275 |
| L9 | 81 | 74 | M | 36 | 82.4 | 51.1 | .1668 | .2500 |
| L10 | 59 | 56 | F | 21 | 84.4 | 60.6 | .0965 | .1550 |
| L11 | 64 | 62 | M | 33 | 86.2 | 42.2 | .1675 | .2275 |
| L12 | 76 | 66 | M | 6 | 77.6 | / | .0390 | / |
| L13 | 79 | 63 | M | 45 | 81.2 | 45.4 | .2068 | .2180 |
| L14 | 84 | 78 | M | 39 | 78.8 | 45.4 | .2420 | .2220 |
| L15 | 72 | 56 | M | 30 | 86.2 | 55.1 | .1743 | .2325 |
| L16** | 76 | / | / | / | 67.9 | / | .1375 | / |
| L17 | 82 | 63 | F | 45 | 86.2 | 11.7 | .1990 | .2313 |
| L18 | 94 | 74 | F | 45 | 88.9 | 58.5 | .2205 | .2615 |
| L19 | 140 | 117 | F | 27 | 94.5 | 69.7 | .2083 | .2228 |
| L20 | 78 | 67 | M | 12 | 88.9 | 67.5 | .1798 | .2120 |
| L21 | 70 | 55 | F | 18 | 78.8 | 60.6 | .2625 | .2850 |
| L22 | 89 | 77 | F | 24 | 65.9 | 72.3 | .2028 | .1975 |
| L23 | 59 | 54 | F | 21 | 78.8 | 51.1 | .1198 | .2053 |
| L24 | 58 | 49 | M | 12 | 81.2 | 60.6 | .1930 | .1185 |
| L25 | 70 | 62 | F | 27 | 87.5 | 58.5 | .2013 | .2200 |
| L26 | 66 | 53 | M | 18 | 78.8 | 55.1 | .1803 | .1623 |
| L27 | 107 | 95 | M | 36 | 86.2 | 56.5 | .2028 | .2250 |
| L28 | 66 | 60 | F | 9 | 83.6 | 66.3 | .1330 | .2130 |
| L29 | 80 | 59 | F | 33 | 78.8 | 60.6 | .1668 | .2375 |
| L30 | 83 | 67 | F | 39 | 88.9 | 53.7 | .1875 | .2340 |
| n | 30 | 27 | 27 | | 30 | 26 | 30 | 26 |
| $\bar{X}$ | 81 | 69 | | | 83.4 | 59.1 | .1836 | .2087 |

*Initial samples were obtained upon arrival of fish from the rearing station. They were then held at the indicated light regime at 16° C during the 45 days of experiment. Final results were obtained from two fish sampled every three days (whenever possible).

**L3, L3, L16 died.

***Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 10

Experiment 3* (Group 3-M, 24L)

| | Weight (g) | | | Time of Sampling (Days) | Molar percent $VP_2$ | | $\Delta D_{max}$*** | |
|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | | Initial | Final | Initial | Final |
| M1* | 80 | / | / | / | / | / | / | / |
| M2 | 91 | 79 | M | 18 | 87.5 | 63.2 | .1870 | .0718 |
| M3 | 91 | 73 | M | 24 | 78.8 | 76.6 | .2025 | .1785 |
| M4 | 83 | 60 | F | 33 | 86.2 | 65.9 | .1365 | .1540 |
| M5 | 75 | 71 | F | 42 | 91.9 | 63.2 | .1895 | .1355 |
| M6 | 74 | 65 | M | 12 | 72.3 | 68.7 | .1750 | .2298 |
| M7 | 34 | 22 | F | 42 | 67.5 | 64.3 | .1753 | .1020 |
| M8 | 95 | 83 | M | 36 | 86.2 | 60.6 | .2050 | .1962 |
| M9 | 74 | 63 | M | 21 | 81.2 | 83.6 | .1665 | .1925 |
| M10 | 77 | 61 | F | 39 | 84.4 | 56.5 | .1290 | .1858 |
| M11 | 85 | 57 | F | 45 | 78.8 | 85.6 | .1990 | .1283 |
| M12 | 79 | 69 | M | 6 | 63.2 | / | .1820 | / |
| M13 | 83 | 65 | F | 27 | 67.5 | 60.6 | .1750 | .1568 |
| M14 | 83 | 70 | M | 33 | 78.8 | 57.2 | .1645 | .1730 |
| M15 | 73 | 62 | F | 9 | 87.5 | 74.1 | .2020 | .1708 |
| M16 | 84 | 69 | M | 9 | 63.2 | 68.7 | .1910 | .1970 |
| M17 | 75 | 70 | F | 30 | 83.6 | 56.6 | .1640 | .1430 |
| M18 | 79 | 70 | M | 24 | 56.5 | 57.2 | .1920 | .1338 |
| M19 | 99 | 86 | F | 36 | 78.8 | 43.2 | .2470 | .1543 |
| M20 | 90 | 74 | F | 15 | 45.4 | 60.6 | .2595 | .1730 |
| M21* | 86 | / | / | / | 91.9 | / | .2120 | / |
| M22 | 70 | 59 | M | 21 | 81.2 | 69.7 | .2550 | .1880 |
| M23 | 77 | 72 | F | 30 | 78.8 | 59.5 | .1560 | .1530 |
| M24 | 70 | 57 | F | 45 | 82.4 | 35.7 | .2330 | .1603 |
| M25 | 90 | 75 | M | 12 | 88.9 | 70.6 | .1590 | .1358 |
| M26 | 86 | 67 | M | 18 | 81.2 | 58.5 | .2550 | .2178 |
| M27 | 83 | 59 | M | 39 | 86.2 | 61.6 | .2420 | .1383 |
| M28 | 89 | 67 | F | 27 | 88.9 | 78.8 | .2400 | .1775 |
| M29 | 63 | 55 | M | 15 | 83.6 | 81.2 | .2380 | .2212 |
| M30 | 70 | 62 | M | 6 | 83.6 | 64.3 | .2389 | .0423 |
| n | 30 | 28 | 28 | | 29 | 27 | 29 | 27 |
| $\bar{X}$ | 70 | 62 | | | 81.5 | 64.5 | .2173 | .1682 |

*Initial samples were obtained upon arrival of fish from the rearing station. They were then held at the indicated light regime at 16° C during the 45 days of experiment. Final results were obtained from two fish sampled every three days (whenever possible).

**M1 died.

***Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 11

Experiment 3* (Group 3-N, 24D)

| | Weight (g) | | | Time of Sampling (Days) | Molar percent $VP_2$ | | $\Delta D_{max}$** | |
|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | | Initial | Final | Initial | Final |
| N1 | 95 | 81 | F | 33 | 75.2 | 25.2 | .1990 | .2668 |
| N2 | 115 | 94 | M | 24 | 74.1 | 29.5 | .2425 | .2618 |
| N3 | 81 | 71 | M | 27 | 53.7 | 14.8 | .2668 | .3135 |
| N4 | 108 | 92 | M | 12 | 63.2 | 39.2 | .2108 | .2373 |
| N5 | 100 | 92 | M | 6 | 78.8 | 64.3 | .2035 | .2358 |
| N6 | 99 | 91 | M | 9 | 55.1 | 45.4 | .2195 | .2685 |
| N7 | 91 | 83 | F | 12 | 78.8 | 49.3 | .2643 | .3488 |
| N8 | 102 | 86 | F | 24 | 74.1 | 29.8 | .2210 | .2850 |
| N9 | 101 | 85 | M | 39 | 69.7 | 22.9 | .2410 | .2970 |
| N10 | 101 | 86 | M | 18 | 72.3 | 35.7 | .1763 | .2580 |
| N11 | 108 | 81 | F | 42 | 82.4 | 22.9 | .1563 | .3180 |
| N12 | 94 | 86 | F | 3 | 70.3 | 74.1 | .2413 | .2858 |
| N13 | 83 | 68 | M | 30 | 77.6 | 39.2 | .1848 | .1843 |
| N14 | 131 | 109 | F | 42 | 72.3 | 9.1 | .1413 | .3720 |
| N15 | 82 | 74 | F | 36 | 81.2 | 9.5 | .2078 | .4023 |
| N16 | 91 | 65 | M | 36 | 53.7 | 18.7 | .2610 | .4585 |
| N17 | 85 | 72 | F | 27 | 82.4 | 42.2 | .1743 | .2053 |
| N18 | 103 | 95 | M | 3 | 74.1 | 70.6 | .2050 | .2833 |
| N19 | 88 | 78 | M | 9 | 83.6 | 65.9 | .2343 | .2877 |
| N20 | 94 | 75 | M | 33 | 86.2 | 40.3 | .1455 | .2735 |
| N21 | 102 | 92 | F | 6 | 86.2 | 75.2 | .1928 | .2765 |
| N22 | 83 | 74 | M | 21 | 76.6 | 37.2 | .1500 | .2960 |
| N23 | 94 | 79 | M | 21 | 70.6 | 27.7 | .2188 | .2550 |
| N24 | 97 | 79 | F | 30 | 78.8 | 29.8 | .2135 | .4170 |
| N25 | 100 | 88 | M | 15 | 78.8 | 39.2 | .1958 | .6113 |
| N26 | 95 | 82 | M | 15 | 78.8 | 35.7 | .2058 | .3610 |
| N27 | 98 | 79 | F | 18 | 72.3 | 53.7 | .2078 | .2585 |
| N28 | 85 | 65 | M | 39 | 48.4 | 25.9 | .2050 | .3775 |
| n | 28 | 26 | 28 | | 28 | 28 | 28 | 28 |
| $\bar{X}$ | 97 | 82 | | | 73.2 | 38.2 | .2066 | .3055 |

*Initial samples were obtained upon arrival of fish from the rearing station. They were then held at the indicated light regime at 16° C during the 45 days of experiment. Final results were obtained from two fish sampled every three days (whenever possible).

**Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 12

Experiment 4* (Group 4-T, 5° C)

| | Weight (g) | | | Time of Sampling (Days) | Molar percent $VP_2$ | | $\Delta D_{max}$*** | |
|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | | Initial | Final | Initial | Final |
| T1 | 85 | 79 | M | 20 | 81.2 | 83.6 | .1875 | .3790 |
| T2* | 79 | / | / | / | 85.6 | / | .1910 | / |
| T3 | 76 | 70 | F | 18 | 77.6 | 82.4 | .1120 | .1373 |
| T4 | 93 | 88 | F | 3 | 74.1 | 66.3 | .1445 | .2328 |
| T5 | 85 | 81 | M | 3 | 65.9 | 69.7 | .1675 | .2238 |
| T6 | 68 | 63 | M | 21 | 75.2 | 72.3 | .1400 | .1525 |
| T7 | 73 | 66 | F | 21 | 87.5 | 78.8 | .1820 | .1523 |
| T8** | 88 | / | / | / | 64.0 | / | .1921 | / |
| T9** | 91 | / | / | / | 59.2 | / | .1675 | / |
| T10 | 87 | 81 | M | 9 | 88.9 | 91.9 | .0845 | .1990 |
| T11 | 105 | 99 | F | 9 | 74.1 | 78.8 | .1475 | .2005 |
| T12 | 83 | 75 | F | 18 | 76.6 | 72.8 | .1338 | .2320 |
| T13** | 68 | / | / | / | 83.8 | / | .1375 | / |
| T14** | 81 | / | / | / | 85.6 | / | .2313 | / |
| T15 | 87 | 84 | M | 6 | 82.4 | 78.8 | .1690 | .2235 |
| T16** | 92 | / | / | / | 66.4 | / | .2960 | / |
| T17** | 76 | / | / | / | 79.0 | / | .1993 | / |
| T18** | 91 | / | / | / | 73.3 | / | .2350 | / |
| T19** | 77 | / | / | / | 82.2 | / | .1940 | / |
| T20** | 86 | / | / | / | 74.5 | / | .2140 | / |
| T21 | 98 | 93 | M | 6 | 93.3 | 83.6 | .1680 | .1860 |
| T22 | 98 | 88 | F | 22 | 94.5 | 78.8 | .1630 | .2280 |
| T23 | 85 | 81 | M | 23 | 81.2 | 82.4 | .2030 | .1750 |
| T24** | 85 | / | / | / | 82.2 | / | .1960 | / |
| T25** | 71 | / | / | / | 57.1 | / | .1970 | / |
| T26 | 94 | 86 | M | 12 | 91.9 | 83.6 | .1160 | .2395 |
| T27 | 82 | 70 | M | 42 | 78.8 | 63.2 | .2095 | .2695 |
| T28 | 90 | 82 | F | 12 | 69.7 | 72.3 | .1315 | .2130 |
| T29 | 94 | 84 | M | 15 | 78.8 | 81.2 | .1765 | .1703 |
| T30 | 62 | 55 | F | 15 | 77.6 | 72.3 | .1545 | .1595 |
| T31** | 84 | / | / | / | 80.0 | / | .1980 | / |
| n | 31 | 18 | 18 | | 31 | 18 | 31 | 18 |
| $\bar{X}$ | 84 | 79 | | | 78.1 | 77.4 | .1770 | .1990 |

*Fish were preconditioned at 4-5° C in laboratory condition for one month before the initial samples were obtained. They were then subjected to the indicated temperature regime under continuous total darkness during the 45 days of experiment. Final results were obtained from two fish sampled every three days (whenever possible).

**Fish died.

***Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 13

Experiment 4* (Group 4-U, 10° C)

| | Weight (g) | | | Days of Sampling (Days) | Molar percent $VP_2$ | | $\Delta D_{max}$*** | |
|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | | Initial | Final | Initial | Final |
| U1 | 82 | 78 | M | 21 | 86.2 | 70.6 | .1600 | .1730 |
| U2 | 79 | 72 | M | 21 | 78.8 | 81.1 | .2180 | .2000 |
| U3 | 80 | 74 | M | 9 | 76.6 | 76.6 | .1995 | .2500 |
| U4 | 72 | 64 | F | 18 | 81.2 | 50.0 | .1640 | .2475 |
| U5 | 74 | 66 | F | 6 | 67.5 | 72.3 | .2040 | .2045 |
| U6 | 88 | 79 | F | 15 | 83.6 | 57.2 | .1910 | .0713 |
| U7 | 104 | 98 | M | 3 | 86.2 | 88.9 | .2220 | .2878 |
| U8** | 72 | / | / | / | 65.8 | / | .0905 | / |
| U9 | 84 | 74 | M | 18 | 76.6 | 69.7 | .1900 | .2745 |
| U10 | 96 | 84 | M | 12 | 55.1 | 56.5 | .1200 | .2075 |
| U11 | 86 | 74 | M | 39 | 87.5 | 49.3 | .1750 | .2398 |
| U12 | 114 | 96 | F | 33 | 83.6 | 66.3 | .1050 | .2763 |
| U13** | 74 | / | / | / | 65.8 | / | .1630 | / |
| U14 | 87 | 78 | F | 12 | 68.7 | 58.6 | .1880 | .2410 |
| U15 | 96 | 78 | F | 24 | 81.2 | 81.2 | .1980 | .2600 |
| U16 | 90 | 82 | F | 3 | 78.8 | 78.8 | .1560 | .1949 |
| U17 | 86 | 75 | F | 15 | 74.1 | 69.7 | .0975 | .2035 |
| U18 | 82 | 70 | M | 24 | 86.2 | 58.5 | .1550 | .2215 |
| U19 | 110 | 91 | M | 33 | 82.4 | 42.2 | .1840 | .1488 |
| U20 | 109 | 95 | M | 27 | 81.2 | 67.5 | .1750 | .2558 |
| U21 | 81 | 70 | M | 45 | 81.2 | 44.7 | .1410 | .2325 |
| U22 | 83 | 71 | F | 27 | 75.2 | 51.1 | .1665 | .1610 |
| U23 | 71 | 58 | M | 45 | 78.8 | 48.4 | .1390 | .2303 |
| U24 | 87 | 75 | M | 6 | 77.6 | 78.8 | .0660 | .1993 |
| U25 | 80 | 69 | M | 39 | 83.6 | 65.9 | .1490 | .2410 |
| U26 | 99 | 88 | M | 9 | 87.5 | 83.6 | .1980 | .1938 |
| U27** | 103 | / | / | / | 85.6 | / | .1360 | / |
| n | 27 | 24 | 24 | | 27 | 24 | 27 | 24 |
| $\bar{X}$ | 88 | 77 | | | 78.1 | 67.3 | .1648 | .2173 |

*Fish were preconditioned at 4-5° C in laboratory condition for one month before the initial samples were obtained. They were then subjected to the indicated temperature regime under continuous total darkness during the 45 days of experiment. Final results were obtained from two fish sampled every three days (whenever possible).

**U8, U13, U27 died.

***Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

## Table 14

### Experiment 4* (Group 4-K, 15° C)

| | Weight (g) | | | | Molar percent $VP_2$ | | $\Delta D_{max}$*** | |
|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | Time of Sampling (Days) | Initial | Final | Initial | Final |
| K1 | 66 | 61 | M | 3 | 48.4 | 57.2 | .0130 | .3248 |
| K2 | 86 | 74 | M | 12 | 69.7 | 44.7 | .2950 | .2585 |
| K3** | 102 | / | / | / | 79.0 | / | .1215 | / |
| K4 | 103 | 85 | M | 30 | 90.4 | 34.4 | .2028 | .2720 |
| K5 | 95 | 86 | M | 12 | 87.5 | 56.5 | .2265 | .2663 |
| K6 | 113 | 99 | F | 3 | 82.4 | 88.9 | .1975 | .2720 |
| K7 | 104 | 100 | F | 9 | 31.5 | 75.2 | .1295 | .0990 |
| K8 | 103 | 96 | M | 6 | 78.8 | 64.3 | .1980 | .2556 |
| K9 | 100 | 81 | F | 36 | 75.2 | 49.2 | .2175 | .2635 |
| K10 | 108 | 86 | M | 24 | 86.2 | 45.4 | .2400 | .3215 |
| K11 | 99 | 87 | M | 24 | 83.6 | 25.9 | .2255 | .3123 |
| K12 | 107 | 88 | M | 18 | 91.9 | 34.4 | .1480 | .2070 |
| K13 | 103 | 82 | M | 30 | 83.6 | 37.2 | .2430 | .2570 |
| K14 | 106 | 88 | M | 18 | 78.8 | 29.8 | .1865 | .2575 |
| K15 | 99 | 75 | F | 42 | 59.5 | 25.9 | .1598 | .2500 |
| K16 | 112 | 105 | F | 6 | 60.6 | 43.2 | .2090 | .2525 |
| K17 | 86 | 76 | M | 9 | 57.2 | 14.7 | .2020 | .2938 |
| K18 | 101 | 87 | M | 15 | 78.8 | 61.6 | .1955 | .3153 |
| K19 | 116 | 100 | F | 15 | 82.4 | 53.7 | .1210 | .2500 |
| K20 | 102 | 83 | F | 36 | 60.6 | 51.1 | .1250 | .2608 |
| K21 | 98 | 80 | F | 42 | 91.9 | 16.8 | .1955 | .2873 |
| K22** | 98 | / | / | / | 79.0 | / | .2070 | / |
| K23 | 102 | 83 | F | 42 | 91.9 | 39.2 | .1520 | .3740 |
| K24 | 96 | 84 | M | 21 | 70.6 | 18.1 | .1740 | .0903 |
| K25 | 101 | 87 | F | 21 | 68.7 | 27.7 | .1470 | .2538 |
| n | 25 | 23 | 23 | | 25 | 23 | 25 | 23 |
| $\bar{X}$ | 100 | 86 | | | 76.5 | 41.4 | .1807 | .3649 |

*Fish were preconditioned at 4-5° C in laboratory condition for one month before the initial samples were obtained. They were then subjected to the indicated temperature regime under continuous total darkness during the 45 days of experiment. Final results were obtained from two fish sampled every three days (whenever possible).

**K3, K22 died.

***Maximum change of optical density units.

('/' means no result available owing either to mortality or unsuccessful determination.)

## Table 15

Experiment 5* (Group 5-P, 12L/12D, 16° C, 26 days)

| Code | Weight (g) |  | Sex | $[T_4]^1$ | Molar percent $VP_2$ |  | $\Delta D_{max}{}^2$ |  | Percent 3-dehydroretinol |  | $[\text{Retinol}]^3$ |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | Initial | Final |  | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| P1 | 86 | 71 | F | / | 96.0 | 60.6 | .1720 | .1878 | 67.0 | / | 37.0 | 59.0 |
| P2 | 101 | 87 | F | 0.18 | 90.4 | 69.7 | .1780 | .2113 | 40.8 | 25.5 | 103.0 | 40.1 |
| P3 | 108 | 85 | M | 0.10 | 94.5 | 83.6 | .2040 | .2203 | 25.5 | 24.3 | 146.3 | 113.9 |
| P4** | 95 | / | / | / | 91.9 | / | .1795 | / | 38.7 | / | 34.0 | / |
| P5 | 92 | 65 | M | 0.22 | 94.5 | 66.3 | .1595 | .1995 | 23.3 | 21.5 | 85.0 | 98.4 |
| P6 | 139 | 115 | F | 0.32 | 88.9 | 49.3 | .2185 | .1789 | 53.1 | 26.9 | 67.9 | 179.8 |
| P7 | 86 | 71 | M | 0.37 | 90.4 | 58.5 | .1390 | .1175 | 36.6 | 20.6 | 55.0 | 172.0 |
| P8 | 89 | 63 | M | / | 91.9 | 66.3 | .1570 | .1573 | 49.6 | 17.4 | 81.3 | 133.7 |
| P9 | 89 | 65 | F | 0.26 | 94.5 | 91.9 | .1710 | .1553 | 61.3 | 13.7 | 70.7 | 149.6 |
| P10 | 97 | 77 | F | 0.51 | 94.5 | 70.6 | .2095 | .1770 | 44.8 | 23.8 | 45.9 | 160.5 |
| P11 | 112 | 92 | M | 0.12 | 94.5 | 66.3 | .1875 | .2160 | 26.9 | 8.2 | 136.0 | 158.2 |
| P12 | 117 | 95 | F | 0.18 | 91.9 | 65.9 | .2410 | .2123 | 43.6 | 15.1 | 55.8 | 171.3 |
| n | 12 | 11 | 11 | 9 | 12 | 11 | 12 | 11 | 12 | 10 | 12 | 11 |
| $\bar{X}$ | 101 | 71 |  | 0.25 | 92.8 | 68.1 | .1847 | .1848 | 42.6 | 19.7 | 76.5 | 125.8 |

*Initial samples were obtained upon arrival of fish from the rearing station. They were then subjected to the indicated light and temperature regimes for a period of 26 to 29 days before the final samples were obtained.

**P4 died.

[1] $[T_4]$ in μg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

[3] [Retinol] in μg retinol/100 ml plasma.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 16

Experiment 5* (Group 5-Q, 12L/12D, 6° C, 29 days)

| | Weight (g) | | | $[T_4]$[1] | Molar percent $VP_2$ | | $\Delta D_{max}$[2] | | Percent 3-dehydroretinol | | [Retinol][3] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| Q1 | 98 | 99 | F | 0.71 | 88.9 | 78.8 | .2190 | .1475 | 18.4 | 17.8 | 181.9 | 134.3 |
| Q2 | 80 | 71 | F | 0.81 | 91.9 | 75.2 | .1870 | .1370 | 34.2 | 12.7 | 96.3 | 183.5 |
| Q3 | 113 | 102 | F | 0.71 | 91.9 | 90.4 | .1530 | .1470 | 46.8 | 11.2 | 55.7 | 126.0 |
| Q4 | 91 | 76 | M | 0.49 | 88.9 | 94.5 | .0970 | .1325 | 51.5 | 13.2 | 52.3 | 160.8 |
| Q5 | 90 | 85 | F | 0.70 | 94.5 | 84.4 | .1380 | .0565 | 24.8 | / | 25.7 | 146.4 |
| Q6 | 118 | 107 | F | 1.17 | 84.4 | / | .1860 | / | 30.8 | 7.8 | 157.6 | 186.2 |
| Q7 | 99 | 88 | M | 0.63 | 90.4 | 83.6 | .2055 | .1610 | 28.7 | / | 124.0 | 109.8 |
| Q8 | 118 | 106 | M | 1.06 | 90.4 | 84.4 | .1820 | .1540 | 25.9 | 6.1 | 184.4 | 145.0 |
| Q9 | 90 | 81 | F | 0.81 | / | 91.9 | / | .1470 | 25.2 | 29.3 | 58.8 | 101.7 |
| Q10** | 95 | / | / | / | 91.9 | / | .2110 | / | 0.0 | / | 108.3 | / |
| Q11 | 85 | 73 | M | 0.36 | 90.4 | 83.6 | .2180 | .1725 | 0.0 | 10.5 | 98.3 | 117.8 |
| Q12 | 94 | 86 | M | 1.07 | 82.4 | 87.5 | .1075 | .1765 | 11.0 | 0.0 | 73.9 | 144.8 |
| n | 12 | 11 | 11 | 11 | 11 | 10 | 11 | 10 | 12 | 9 | 12 | 11 |
| $\bar{X}$ | 98 | 88 | | 0.77 | 89.6 | 85.4 | .1731 | .1420 | 24.8 | 12.1 | 105.6 | 141.5 |

*Initial samples were obtained upon arrival of fish from the rearing station. They were then subjected to the indicated light and temperature regimes for a period of 26 to 29 days before the final samples were obtained.

**Q10 died.

[1] $[T_4]$ in μg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

[3] [Retinol] in μg/100 ml plasma.

('/' means no result available owing either to mortality or unsuccessful determination.)

## Table 17

Experiment 5* (Group 5-R, 24D, 16° C, 26 days)

| Code | Weight (g) | | Sex | $[T_4]^1$ | Molar percent $VP_2$ | | $\Delta D_{max}{}^2$ | | Percent 3-dehydroretinol | | $[\text{Retinol}]^3$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | Final | | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| R1 | 77 | 57 | F | 1.59 | 93.3 | 66.3 | .1240 | .3170 | 16.6 | 20.2 | 178.0 | 132.5 |
| R2 | 106 | 88 | M | 0.77 | 91.9 | 42.2 | .2070 | .3100 | 0.0 | 14.5 | 161.9 | 229.8 |
| R3** | 114 | / | / | / | 90.4 | / | .1445 | / | 22.0 | / | 173.0 | / |
| R4 | 86 | 73 | F | 0.57 | 87.5 | 35.7 | .1640 | .2780 | 7.14 | 14.6 | 82.2 | 179.6 |
| R5 | 87 | 72 | F | 0.57 | 93.3 | 44.7 | .2310 | .2860 | 17.2 | 15.7 | 129.8 | 117.8 |
| R6 | 85 | 72 | M | 0.33 | 93.3 | 58.5 | .1780 | .2570 | 24.9 | 8.7 | 86.9 | 189.5 |
| R7 | 96 | 80 | F | 0.72 | 94.5 | 42.2 | .2115 | .2020 | 20.2 | 7.4 | 109.9 | 186.4 |
| R8 | 93 | 74 | M | 1.01 | 94.5 | 32.3 | .2340 | .2410 | 0.0 | 9.3 | 71.0 | 102.0 |
| R9 | 100 | 74 | M | 0.42 | 94.5 | 48.4 | .1840 | .2120 | 26.2 | 14.2 | 140.9 | 129.5 |
| R10 | 92 | 72 | M | 0.00 | 93.3 | 22.9 | .1850 | .2780 | 37.6 | 10.0 | 47.8 | 133.8 |
| R11 | 93 | 79 | F | 0.29 | 93.3 | 29.8 | .1860 | .1580 | 0.0 | 15.6 | 99.9 | 92.8 |
| R12 | 92 | 97 | F | 0.51 | 90.4 | 31.5 | .1420 | .3175 | 18.4 | 15.0 | 92.2 | 109.0 |
| $n$ | 12 | 11 | 11 | 11 | 12 | 11 | 12 | 11 | 12 | 11 | 12 | 11 |
| $\bar{X}$ | 93 | 74 | | 0.61 | 92.5 | 41.3 | .1826 | .2597 | 15.9 | 13.2 | 114.5 | 145.7 |

*Initial samples were obtained upon arrival of fish from the rearing station. They were then subjected to the indicated light and temperature regimes for a period of 26 to 29 days before the final samples were obtained.

**R3 died.

[1] $[T_4]$ in µg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

[3] [Retinol] in µg/100 ml plasma.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 18

Experiment 5* (Group 5-S, 24D, 6° C, 29 days)

| Code | Weight (g) |  | Sex | $[T_4]^1$ | Molar percent $VP_2$ |  | $\Delta D_{max}^2$ |  | Percent 3-dehydroretinol |  | $[\text{Retinol}]^3$ |  |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | Initial | Final |  | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
| S1 | 113 | 104 | M | 1.04 | 88.9 | 67.5 | .2120 | .2420 | 0.0 | / | 69.1 | / |
| S2 | 90 | 84 | F | 1.19 | 91.9 | 68.7 | .1830 | .1587 | 35.1 | / | 94.2 | / |
| S3 | 93 | 88 | F | 0.89 | 93.3 | / | .1405 | / | 0.0 | 3.4 | 109.6 | 203.7 |
| S4 | 84 | 77 | M | 0.65 | 82.4 | 72.3 | .2250 | .1718 | 0.0 | 0.0 | 47.1 | 181.7 |
| S5 | 85 | 79 | F | 0.65 | 94.5 | 77.6 | .2000 | .1658 | 30.6 | 10.8 | 61.3 | 158.5 |
| S6 | 96 | 98 | F | 0.69 | 93.3 | 64.3 | .2190 | .2310 | 30.2 | 11.8 | 60.4 | 241.7 |
| S7 | 83 | 74 | M | 0.87 | 93.3 | 77.6 | .2222 | .2500 | 21.7 | 0.0 | 73.7 | 103.8 |
| S8 | 92 | 83 | M | 0.44 | 90.4 | 81.2 | .1160 | .2070 | 0.0 | 0.0 | 79.9 | 93.0 |
| S9 | 74 | 68 | M | 0.00 | 83.6 | 49.3 | .2050 | .3003 | 51.5 | 9.1 | 45.9 | 149.6 |
| S10 | 86 | 81 | M | 0.78 | 93.3 | 61.6 | .0675 | .2028 | 40.4 | 12.5 | 43.3 | 119.5 |
| S11 | 84 | 77 | F | 0.32 | 91.9 | 66.3 | .1695 | .2625 | 0.0 | 8.7 | 37.6 | 167.7 |
| S12 | 92 | 85 | M | 0.06 | 93.3 | 63.2 | .1730 | .1808 | 23.7 | 14.6 | 65.8 | 206.8 |
| n | 12 | 12 | 12 | 12 | 12 | 11 | 12 | 11 | 12 | 10 | 12 | 10 |
| $\bar{X}$ | 89 | 82 |  | 0.63 | 90.8 | 68.1 | .1770 | .2156 | 19.4 | 7.1 | 65.8 | 162.6 |

*Initial samples were obtained upon arrival of fish from the rearing station. They were then subjected to the indicated light and temperature regimes for a period of 26 to 29 days before the final samples were obtained.

[1] $[T_4]$ in μg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

[3] [Retinol] in μg/100 ml plasma.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 19

Experiment 6* (Group 6-E, 24D, control)

| Code | Weight (g) |  | Sex | $[T_4]$[1] |  | Molar percent $VP_2$ |  | $\Delta D_{max}$[2] |  | Percent 3-dehydroretinol | [Retinol][3] |
|---|---|---|---|---|---|---|---|---|---|---|---|
|  | Initial | Final |  | Initial | Final | Initial | Final | Initial | Final | Final | Final |
| G8 | 104 | 100 | F | 0.39 | 0.80 | 16.8 | 18.1 | .2848 | .4060 | 0.0 | 142.6 |
| G5 | 115 | 121 | M | 1.05 | 0.24 | 22.9 | 27.7 | .3248 | .2988 | 4.9 | 254.5 |
| H2 | 89 | 84 | M | 0.20 | 0.50 | 44.7 | 44.7 | .3495 | .2620 | 12.7 | 249.5 |
| G1 | 102 | 94 | F | 0.89 | 0.16 | 27.7 | 35.7 | .4313 | .3705 | 9.1 | 233.2 |
| H7 | 88 | 81 | F | 0.52 | 0.40 | 25.9 | 25.9 | .4350 | .3733 | 0.0 | 112.3 |
| G6 | 84 | 79 | M | 0.63 | 0.00 | 9.5 | 18.1 | .3253 | .2328 | 0.0 | 161.8 |
| n | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| $\bar{X}$ | 97 | 93 | 0.61 |  | 0.35 | 24.6 | 28.4 | .3431 | .3239 | 4.3 | 192.3 |

*Fish were preconditioned at 16° C under continuous total darkness for 30 days before the initial samples were taken. They were then subjected to 8° C for 10 days under the indicated experimental condition before the final samples were obtained.

[1] $[T_4]$ in µg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

[3] [Retinol] in µg/100 ml plasma.

Table 20

Experiment 6* (Group 6-F, 24D, thyroxine introduced to tank water at a concentration of 10 μg/100 ml)

| | Weight (g) | | | $[T_4]$[1] | | Molar percent $VP_2$ | | $\Delta D_{max}$[2] | | Percent 3-dehydroretinol | [Retinol][3] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Code | Initial | Final | Sex | Initial | Final | Initial | Final | Initial | Final | Final | Final |
| F1 | 86 | 76 | F | 0.18 | 2.96 | 16.8 | 47.4 | .3543 | .2973 | 15.0 | 146.8 |
| F2 | 98 | 88 | M | 0.52 | 2.80 | 14.8 | 53.7 | .4060 | .3018 | 8.6 | 130.0 |
| F3** | 88 | / | / | 0.06 | / | / | / | / | / | / | / |
| F4 | 96 | 87 | F | 0.38 | 3.62 | 21.6 | 58.5 | .3523 | .2775 | 4.4 | 156.0 |
| F5 | 112 | 99 | F | 0.31 | 2.96 | 35.7 | 58.5 | .3018 | .2945 | 0.0 | 171.7 |
| F6 | 78 | 67 | F | 0.25 | 3.50 | 18.8 | 44.7 | .3695 | .3532 | 8.0 | 185.3 |
| F7** | 92 | / | / | 0.74 | / | / | / | / | / | / | / |
| F8 | 79 | 66 | F | 0.54 | 2.19 | 43.2 | 67.5 | .2345 | .2673 | 11.6 | 159.0 |
| F9** | 94 | / | / | 0.48 | / | 5.5 | / | .2843 | / | / | / |
| F10 | 96 | 87 | M | 0.19 | 3.93 | 25.9 | 51.1 | .2553 | .3745 | 0.0 | 169.3 |
| n | 10 | 7 | 7 | 10 | 7 | 8 | 7 | 8 | 7 | 7 | 7 |
| $\bar{X}$ | 92 | 81 | | 0.37 | 3.1 | 20.6 | 54.5 | .3198 | .3096 | 6.8 | 159.6 |

*Fish were preconditioned at 16° C under continuous total darkness for 30 days before the initial samples were taken. They were then subjected to 8° C for 10 days under the indicated experimental condition before the final samples were obtained.

**F3, F7, F9 died.

[1] $[T_4]$ in μg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

[3] [Retinol] in μg/100 ml plasma.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 21

Experiment 6* (Group 6-G, 12L/12D, control)

| Code | Weight (g) |  | Sex | $[T_4]^1$ |  | Molar percent $VP_2$ |  | $\Delta D_{max}^2$ |  | Percent 3-dehydroretinol | $[\text{Retinol}]^3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
|  | Initial | Final |  | Initial | Final | Initial | Final | Initial | Final | Final | Final |
| G2 | 83 | 75 | F | 0.70 | 0.00 | 5.5 | 51.1 | .2593 | .1920 | / | / |
| G4 | 68 | 66 | F | 0.87 | 0.03 | 31.5 | 78.8 | .3788 | .1808 | 0.0 | 62.5 |
| G7 | 126 | 118 | M | 0.31 | 0.16 | 14.8 | / | .2000 | / | 14.4 | 152.8 |
| G9 | 112 | 105 | F | 1.03 | 0.30 | 9.5 | 53.7 | .3843 | .1995 | 3.14 | 189.8 |
| G10 | 89 | 86 | M | 0.79 | 0.06 | 32.3 | 78.8 | .2675 | .1708 | 0.0 | / |
| G11 | 97 | 88 | F | 0.82 | 0.00 | / | 65.9 | / | .2655 | / | / |
| G12 | 83 | 90 | M | 0.76 | 0.00 | / | 78.8 | / | .2288 | / | / |
| G14 | 84 | 86 | F | 0.91 | 0.75 | / | 72.8 | / | .3065 | / | / |
| n | 8 | 8 | 8 | 8 | 8 | 5 | 7 | 5 | 7 | 4 | 4 |
| $\bar{X}$ | 93 | 89 |  | 0.77 | 0.16 | 18.7 | 65.8 | .2980 | .2174 | 4.5 | 135.0 |

*Fish were preconditioned at 16° C under continuous total darkness for 30 days before the initial samples were taken. They were then subjected to 8° C for 10 days under the indicated experimental condition before the final samples were obtained.

[1] $[T_4]$ in µg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

[3] [Retinol] in µg/100 ml plasma.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 22

Experiment 6* (Group 6-H, 12L/12D, thyroxine introduced to tank water at a concentration of 10 μg/100 ml)

| Code | Weight (g) |  | Sex | $[T_4]$[1] |  | Molar percent $VP_2$ |  | $\Delta D_{max}$[2] |  | Percent 3-dehydroretinol | [Retinol][3] |
|---|---|---|---|---|---|---|---|---|---|---|---|
|  | Initial | Final |  | Initial | Final | Initial | Final | Initial | Final | Final | Final |
| H3 | 97 | 92 | M | 0.44 | 4.15 | 47.4 | 94.5 | .4112 | .2128 | 25.9 | 69.6 |
| H4 | 68 | 65 | M | 0.27 | 5.55 | 14.8 | 84.4 | .3995 | .2260 | 9.9 | 125.7 |
| H5 | 90 | 84 | M | 0.64 | 2.07 | 9.1 | 72.8 | .3393 | .1633 | 19.7 | 56.4 |
| H6 | 77 | 72 | F | 0.28 | 2.19 | 22.9 | 90.4 | .2525 | .1470 | 26.3 | 51.3 |
| H9 | 81 | 78 | M | 0.52 | 3.70 | 27.7 | 84.4 | .3145 | .1760 | 0.0 | 115.8 |
| H10 | 70 | 66 | M | 0.38 | 4.27 | 14.8 | 83.6 | .3180 | .1865 | 15.8 | 102.0 |
| H11 | 94 | 87 | F | 0.72 | 3.98 | / | 86.2 | / | .2098 | / | / |
| H12 | 93 | 87 | M | 0.58 | 5.10 | / | 86.2 | / | .1463 | / | / |
| H15 | 86 | 77 | M | 0.83 | 2.35 | / | 70.6 | / | .1848 | / | / |
| n | 9 | 9 | 9 | 9 | 9 | 6 | 9 | 6 | 9 | 6 | 6 |
| $\bar{X}$ | 84 | 79 |  | 0.52 | 3.70 | 22.8 | 83.7 | .3391 | .1863 | 16.3 | 67.2 |

*Fish were preconditioned at 16° C under continuous total darkness for 30 days before the initial samples were taken. They were then subjected to 8° C for 10 days under the indicated experimental condition before the final samples were obtained.

[1] $[T_4]$ in μg thyroxine/100 ml plasma.

[2] Maximum change of optical density units.

[3] [Retinol] in μg/100 ml plasma.

('/' means no result available owing either to mortality or unsuccessful determination.)

Table 23

Experiment 7

| Code | Weight (g) |  | Sex | Time of Sampling (Days) | Molar percent $VP_2$ |  | $\Delta D_{max}$*** |  | Difference (Molar percent $VP_2$) |
|---|---|---|---|---|---|---|---|---|---|
|  | Initial | Final |  |  | Left eye | Right eye | Left eye | Right eye |  |
| 411 | 73 | 78 | M | 3 | 51.1(T)** | 43.2(B)** | .1575 | .1653 | 7.9 |
| 414 | 71 | 73 | F | 4 | 32.3(T) | 18.1(B) | .2410 | .2535 | 14.2 |
| 415 | 99 | 101 | M | 4 | 34.4(T) | 27.7(B) | .2878 | .1895 | 6.7 |
| 422 | 99 | 99 | F | 4 | 32.3(B) | 29.8(B) | .2790 | .2773 | 2.5 |
| 401 | 73 | 76 | F | 5 | 25.2(B) | 34.4(T) | .2903 | .2768 | 9.2 |
| 426 | 86 | 77 | M | 6 | 44.7(T) | 49.3(T) | .2875 | .2128 | 4.6 |
| 381 | 77 | 68 | M | 10 | 56.5(T) | 72.3(B) | .2663 | .2900 | 15.8 |
| 382 | 90 | 85 | M | 10 | 56.5(B) | 78.8(T) | .2905 | .2570 | 24.3 |
| 384 | 88 | 83 | M | 10 | 48.4(B) | 65.9(T) | .2463 | .2450 | 17.5 |
| 402 | 80 | 70 | F | 10 | 47.4(B) | 72.3(T) | .2788 | .2770 | 24.9 |
| 403 | 84 | 77 | F | 10 | 35.7(B) | 53.7(T) | .3070 | .2070 | 18.0 |
| 404 | 81 | 69 | M | 10 | 42.2(B) | 66.3(T) | .2488 | .1988 | 24.1 |
| 406 | 74 | 72 | M | 10 | 48.4(B) | 60.6(T) | .2443 | .2730 | 12.2 |
| 412 | 82 | 82 | F | 10 | 74.1(T) | 45.4(B) | .0550 | .2443 | 28.7 |
| 421 | 76 | 64 | F | 10 | 49.3(B) | 49.3(B) | .2765 | .2603 | 0.0 |

*Fish were preconditioned at 16° C in continuous total darkness for three weeks before they were individually fitted with black and transparent eye caps as indicated in the table. During the experiment, they were held at 8° C under continuous illumination (24L).

**(T): Eye fitted with transparent cap.
(B): Eye fitted with black cap.

***Maximum change of optical density units.

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